FEB 9 1918
LIBRARY

VOLUME III NUMBER 1

JOURNAL OF BACTERIOLOGY

OFFICIAL ORGAN OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

JANUARY, 1918

EDITOR-IN-CHIEF
C.-E. A. Winslow

It is characteristic of Science and Progress that they continually open new fields to our vision.—Pasteur

PUBLISHED MONTHLY
WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Entered as second-class matter April 2, 1918, at the Post Office at Baltimore, Maryland, under the Act of March 3, 1879

Of Special Current Interest

LABORATORY *and* CLINICAL

Fairchild Culture and Tablet of the Bacillus Bulgaricus, type A—a superior culture, maintained, standardized, by perfected laboratory technic.

Pepton, Fairchild—for all the purposes of the bacteriological and antitoxin laboratory.

Trypsin, Fairchild—isolated to a great degree from the other enzymes and constituents of the pancreas gland, standardized; the most potent product of this order—10,000 Roberts' units. It is not pancreatin labelled "trypsin."

Bile Salts, Fairchild—the sodium glycocholate and taurocholate in their native association, separated from fresh ox gall.

Holadin—the entire pancreas extract; potent in amylopsin, trypsin, lipase, with associated gland constituents; of increasing interest and application in therapeutics; especially serviceable in meagash work.

Holadin and Bile Salts—suggested in view of the "close relationship between the bile and the pancreatic juice" (as shown by Pawlow and others), and of great clinical service.

Gastrosin—an alcoholic, glycerin-acid-aqueous extract of the entire stomach mucosa; contains the enzymes with the associated soluble proteins and inorganic constituents; optimum of enzyme energy.

Enemase—for colonic alimentation. Obtained by physiological conversion of lean beef and whole wheat; the complex of proteins, amino-acids, etc., dextrins and maltose, and all associated soluble and convertible substances, organic and inorganic, extractives, vitamins, stimulating and activating principles of the native raw foods; perfectly diffusible, assimilable. Concentrated, standardized; ready by simple dilution at the moment required.

Fairchild Brothers and Foster

New York

Specialists in the Applied Chemistry of the Digestive Enzymes

JOURNAL OF BACTERIOLOGY

OFFICIAL ORGAN OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

DEVOTED TO THE ADVANCEMENT AND DISSEMINATION OF KNOWLEDGE IN REGARD TO THE BACTERIA AND OTHER MICRO-ORGANISMS

Editorial Board

Editor-in-Chief

C.-E. A. WINSLOW

Yale Medical School, New Haven, Conn.

A. PARKER HITCHENS — R. E. BUCHANAN, Ex officio

Advisory Editors

C. C. BASS
R. E. BUCHANAN
P. F. CLARK
F. P. GAY
F. P. GORHAM
F. C. HARRISON
H. W. HILL
E. O. JORDAN
A. I. KENDALL
C. B. LIPMAN
C. E. MARSHALL
V. A. MOORE
M. E. PENNINGTON
E. B. PHELPS
L. F. RETTGER
L. A. ROGERS
M. J. ROSENAU
W. T. SEDGWICK
F. L. STEVENS
A. W. WILLIAMS
H. ZINSSER

CONTENTS

OSCAR TEAGUE. The Toxicity of Victoria Blue 4 R. for B. Paratyphosus A., B. Paratyphosus B. and B. Enteriditis 1

IVAN C. HALL. Automatic Water Level for Arnold Sterlizers 7

E. M. MEYER. An Aerobic Spore-Forming Bacillus Giving Gas in Lactose Broth Isolated in Routine Water Examination 9

PAUL W. ALLEN. A Simple Method for the Classification of Bacteria as to Diastase Production 15

KAN-ICHIRO MORISHIMA. A Simple and Practical Medium for Differentiating B. Typhosis, B. Paratyphosus A., B. Paratyphosus B., B. Enteritidis, and B. Coli .. 19

R. G. PERKINS. A New Apparatus for Obtaining Simultaneous Cultures of any Desired Age for Comparative Study 23

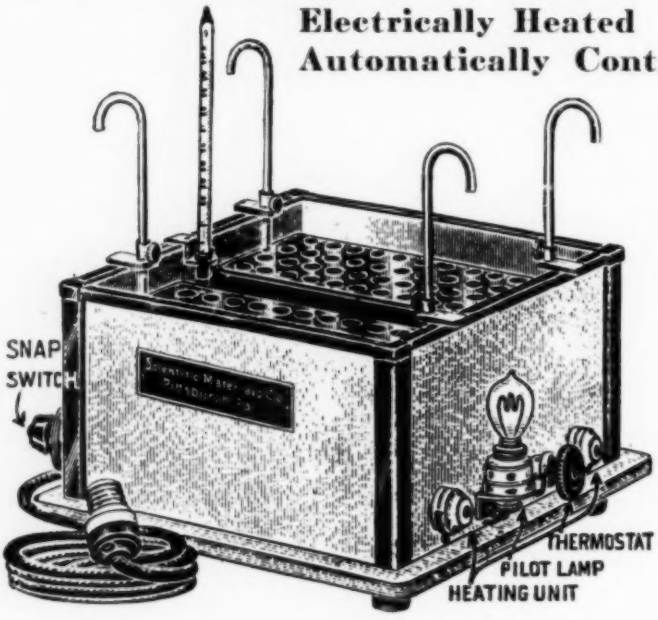
R. E. BUCHANAN. Studies in the Nomenclature and Classification of the Bacteria. V. Subgroups and Genera of the Bacteriaceae 27

FRED W. TANNER. A Study of Green Fluorescent Bacteria from Water 63

Abstracts of American and foreign bacteriological literature appears in a separate journal, *Abstracts of Bacteriology*, published bi-monthly by the Williams & Wilkins Company, under the editorial direction of the Society of American Bacteriologists. Price, $5.00 North America; $5.50 elsewhere.

Wassermann Bath

Electrically Heated
Automatically Controlled



Fulfills the demand for a small and conveniently arranged bath which will maintain a constant temperature. Used for serological work, for holding agar tubes above the solidifying temperature, for standardizing disinfectants, etc.

The bath is constructed of heavy copper on the inside and $\frac{3}{16}$" transite on the outside, with a space between which is filled with animal hair. This insulation enables a constant temperature to be maintained in the bath regardless of the variations in the room temperature.

The special units heating the bath are placed in copper tubes near the bottom of the water and can be very easily replaced. The temperature is controlled by the latest type of electro-thermo regulator placed in a copper tube near these heating units.

The racks are constructed of heavy copper nicely finished. They have adjustable supports which make it possible to immerse them to any desired depth in the bath. They also have convenient handles.

No. 51 Wassermann Bath (80 test tubes capacity), complete with 2 racks, cord and connecting plug **$50.00**

No. 54 Wassermann Bath (40 test tube capacity), complete with one rack, cord and connecting plug **$37.50**

No. 55 Additional Racks for Wassermann Bath. These can be supplied, if desired, at ..... **$5.00 Each**

Scientific Materials Company

PITTSBURGH, PA.

Everything for the Laboratory

INFORMATION FOR CONTRIBUTORS

Although there are numerous journals in the United States that deal with various special phases of bacteriology (as applied to Medicine, Sanitary Science, Agriculture and the like), there has been no journal in the English language to represent the science as a whole.

The Society of American Bacteriologists has established the JOURNAL OF BACTERIOLOGY as its official organ and as a medium for the discussion of the more general problems of the science—the structure and physiology of the microbes, the inter-relationships of microbic types, the effects of physical and chemical agents upon microbic life, the mutual interactions of microbes growing together in various media, the nutritional needs and products of metabolic activity of various microbes, and new methods of laboratory technique—and similar advances in knowledge which are so fundamental as to be of vital interest to workers in all parts of this great field. The JOURNAL OF BACTERIOLOGY includes in its scope not only the bacteria but other related micro-organisms, yeasts, molds, protozoa, etc. While it is planned to make the JOURNAL in particular an organ for the more fundamental and general aspects of bacteriology, it will necessarily include many papers whose interest is mainly technical, particularly in those fields of bacteriology which have now no satisfactory organ of publication at their disposal.

Manuscripts should be sent to Prof. C.-E. A. Winslow, Yale Medical School, New Haven, Conn.

All other communications pertaining to editorial work should be addressed to Dr. A. Parker Hitchens, Glenolden, Pa.

Twenty-five reprints of articles will be furnished gratis to contributors when ordered in advance. A table showing cost, with an order slip, is sent with proof.

INFORMATION FOR SUBSCRIBERS

THE JOURNAL OF BACTERIOLOGY is issued bi-monthly, in January, March, May, July, September, and November.

A volume consists of 600 pages. Subscriptions to the Journal are taken for the volume only. Under the present plans, one volume is issued a year

Back Volumes can be furnished: Vol. I, 1916, Nos. 1-6 incl., $5.00, domestic; $5.50, foreign. Vol. II, 1917, Nos. 1-6 incl., $5.00, domestic; $5.50 foreign.

Correspondence concerning business matters should be addressed to the Williams & Wilkins Company, 2419-21 Greenmount Avenue, Baltimore, U. S. A.

Subscriptions are received:

For Argentina and Uruguay: Beutelspacher & Cia., Sarmiento 815, Buenos Aires, Argentina.

For the British Empire, except Canada: Mr. C. F. Clay, Manager, The Cambridge University Press, Fetter Lane, London, E. C. Subscription price 23s. net, postpaid.

For Canada: Wm. Dawson & Sons, Ltd., 87 Queen Street East, Toronto, Canada.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11 to 15 Nihonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan. Subscription price, ¥11.50 net, postpaid.

For Italy: Ulrico Hoepli, Milan, Italy. Subscription price, $5.50 net, postpaid.

For Switzerland: George & Cie, Freiestrasse 10, Basel, Switzerland. Subscription price, $5.50 net, postpaid.

For the United States and other countries except as above: WILLIAMS & WILKINS COMPANY, 2419-2421 GREENMOUNT AVE., BALTIMORE, U. S. A. Subscription price, North America, and U. S. possessions, $5.00 net, postpaid; other countries $5.50 net, postpaid.

Claims for copies lost in the mails will not be allowed unless such claims are received within 30 days (60 days, foreign) of the date of issue. Claimants must state that the publication was not delivered at their recorded address. The publishers will not be responsible for loss due to change of address unless notification is received at least one week in advance of issue.

1917 **Abstracts of Bacteriology** Volume I

Comprising reviews and abstracts of American and foreign work in Bacteriology, Mycology and Protozoology in their relation to the arts and sciences

Under the editorial direction of the
SOCIETY OF AMERICAN BACTERIOLOGISTS

EDITOR
A. PARKER HITCHENS

ASSOCIATE EDITOR
GEORGE H. SMITH

Published bi-monthly. One volume a year at present. About 600 pages to a volume.

Price: { North America, $5.00, a volume, net postpaid.
Foreign, $5.50, a volume, net postpaid.

Subscriptions are received for the volume only. Single copies are not sold

---

Subscription Order for the Abstracts of Bacteriology

WILLIAMS & WILKINS COMPANY
2419-21 GREENMOUNT AVENUE
BALTIMORE, MD., U. S. A.

..... 191....

I / We { enclose { $5.00 (U. S.-Canada) / $5.50 (foreign) } for subscription to Vol. I, ABSTRACTS OF BACTERIOLOGY.

Name .....
(Please state whether Dr., Prof., etc.)

Address .....

Cases for Volume I of The Journal of Bacteriology will be furnished for fifty cents if ordered promptly. Blue Cloth, Stamped in Gold.

Williams & Wilkins Company
2419-21 Greenmount Avenue
Baltimore, - U. S. A.

THE TOXICITY OF VICTORIA BLUE 4 R. FOR B. PARATYPHOSUS A, B. PARATYPHOSUS B AND B. ENTERITIDIS

OSCAR TEAGUE

From the Department of Hygiene, Loomis Laboratory, Cornell Medical College, New York

While studying three years ago the relative toxicity of various dyes on strains of *B. typhi*, *B. paratyphi* A and B and *B. coli*, I noticed a marked difference in the toxicity of Victoria blue 4 R. for *B. paratyphi* A and *B. paratyphi* B. A careful comparison of the growth of a fairly large number of strains of these organisms and of *B. enteritidis*, upon the same lot of agar containing different amounts of the stain, was made and, though the results were clearly defined, viz., the paratyphoid B bacillus being more readily inhibited than paratyphoid A and *B. enteritidis*, yet they did not seem at the time of sufficient importance to warrant their publication.

At present, however, as infections with these organisms have become more common, and a study of their characteristics has been undertaken in a number of American laboratories, it would seem that my observations might possibly supplement some of these studies and should, therefore, be presented.

Meat infusion agar was prepared in the usual way with 1 per cent Witte's peptone and 1/2 per cent sodium chloride in the Arnold sterilizer. It was cleared with egg white, filtered through cotton and titrated to + 1. The medium was sterilized by being heated for three successive days in the Arnold. A stock solution of Victoria blue 4 R. was prepared and the requisite amounts of this were added to comparatively large amounts of the agar to yield 1/20, 1/30, 1/40 and 1/50 per cent respectively of the stains; the required number of plates were poured from each. Hence all the plates containing a given per cent of the stain were uniform in every respect.

For the tests fresh transplants of the cultures[1] were used after twenty-four hours' incubation. Suspensions of the different cultures in saline solution were prepared of approximately the same cloudiness, and 1 loop of an appropriate dilution of each was inoculated upon plates with different percentages of the Victoria blue and upon a control plate containing no stain. The cultures were emulsified and inoculated in lots of four in the order recorded in table 1.

All of the cultures were subjected to the test on the same day, in order to ensure uniformity of conditions. It was not convenient to count the colonies on all the plates at the end of twenty-four hours, so the number was recorded as "numerous" where there was a good growth with obviously little or no inhibition due to the presence of the dye Eighteen cultures of *B. paratyphi* A., 18 of *B. paratyphi* B and 11 cultures of *B. enteritidis* were employed. Some of these may have been duplicates, having been obtained by different laboratories from the same original strain. The paratyphoid A strains without exception gave good growth even on the plates containing the maximum amount of the stain; the colonies were quite large after twenty-four hours' incubation and the reduction in number as compared with the control plates was usually considerably less than 50 per cent. The paratyphoid B cultures, on the other hand, were almost completely inhibited in their growth by 1/20 per cent and 1/30 per cent of Victoria blue and some of them were inhibited also by 1/40 per cent and 1/50 per cent of the dye; the few colonies that did appear were usually quite small after twenty-four hours' incubation. The *B. enteritidis* culture behaved on the plates like the paratyphoid A cultures. This seemed surprising, as they resemble paratyphoid B more closely in most of their characteristics than paratyphoid A.

[1] Stock laboratory cultures of *B. paratyphi A* and *B* and of *B. enteritidis* were obtained from the New York City Health Department Research Laboratory, the Hygienic Laboratory and the United States Army Medical School in Washington, the Mulford Laboratories, the Museum of Natural History, New York, and from the Department of Tropical Medicine at Harvard.

TABLE 1

Victoria blue 4 R

CULTURES	1/20 PER CENT		1/30 PER CENT		1/40 PER CENT		1/50 PER CENT		CONTROL
	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	
B N. Y. Hosp...	0	0	0	3	4	45	0	81	246
B 97.....	0	0	0	2	0	13	0	14	92
A 60.....	f. num.	121	f. num.		f. num.		f. num.		132
B 25.....	0	0	0	3	3	11	3	8	174
B And.....	0	0	0	0	0	0	0	8	271
B 66.....	0	0	0	0	0	0	0	5	119
A 57.....	f. num.	106	f. num.	118	f. num.	92	f. num.	117	218
Gärtner Mt. S...	num.	189	num.		num.		num.		320
A 3.....	f. num.	109	num.		num.		num.		156
B Am.....	0	7	few	46	f. num.	49	num.	100	111
A 56.....	num.	197	num.		num.		num.		310
A 63.....	num.	68	num.	67	num.		num.	76	140
B 1.....	0	0	0	0	0	1	0	1	105
B 59.....	1	7	10	12	20	25	18	23	152
A And.....	num.	60	num.	55	num.	73	num.		92
Schottmüller....	0	0	0	3	few	27	f. num.	37	100
B 52.....	0	0	0	0	0	1	0	2	137
Gärtner And.....	num.	145	num.	171	num.		num.		169
B 2.....	0	0	0	0	0	0	0	1	74
Kurth.....	0	0	0	3	few	21	few	49	103
A 55.....	num.	448	num.	58	num.	48	num.	55	81
A 4.....	num.	59	num.	97	num.	92	num.	86	112
B 65.....	0	0	0	0	0	1	1	5	101
B Boston 1.....	0	0	0	1	few	34	28	40	156
A 54.....	157		num.		num.		num.		186
A 62.....	29	29	30	33	28	28	32	32	75
A 7.....	num.	97	num.		num.		76		146
A 1.....	num.	169	num.		num.		num.		222
B Boston 2.....	3	3	18	18	num.	96	f. num.		175
Enteritidis 69...	num.	260	num.		num.		num.		400
B 61.....	0	3	f. num.	65	num.	76	num.	112	150
A 58.....	num.	85	num.	90	num.	96	num.		150

TABLE 1—Continued

CULTURES	1/20 PER CENT		1/30 PER CENT		1/40 PER CENT		1/50 PER CENT		CONTROL
	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	
A Yale	num.	185	num.		num.		num.		300
Paratyphoid A	num.	100	num.		num.		num.		174
B 53	0	13	f. num.	43	num.	66	num.	66	81
B Roanoke	few	20	f. num.	34	num.	51	num.	62	50
Enteritidis 14	num.	117	num.		num.		num.		124
A 2	num.	67	num.	65	num.	87	num.	94	86
Gärtner 93	num.	71	num.	103	num.		num.		119
A Cafirin	num.	129	num.	104	num.		num.		140
A W. B	num.	110	num.		num.		num.		131
Enteritidis 70	num.	192	num.		num.		num.		161
Enteritidis And.	few	31	few	37	f. num.	66	f. num.	56	127
Enteritidis 47	num.	160	num.		num.		num.		160
Enteritidis 67	num.	148	num.		num.		num.		230
Enteritidis 64	f. num.	100	num.		f. num.		num.		206

num. = numerous colonies.
f. num. = fairly numerous colonies.

Five of the paratyphoid B. cultures (Am. Boston 2, 61, 53, and Roanoke) and 2 of the B. enteritidis cultures (And. and Whit.) gave intermediate results; that is, they were not inhibited as completely as paratyphoid B cultures, nor did they give as good growth as the paratyphoid A cultures. The paratyphoid organisms are known to undergo mutations occasionally after having been grown on artificial culture media for long periods of time, the B type assuming characteristics of the A type and vice versa. It seemed not unlikely that these few cultures may have behaved atypically with regard to growth on the Victoria blue agar because such mutations had occurred in them. Another possibility is that they may have had their source from some of the lower animals and not from infected human beings.

Similar experiments were performed with cultures of *B. suipestifer*, *B. typhi murium* and *B. Danysz*, the results of which are recorded in table 2.

TABLE 2

Victoria blue 4 R

CULTURE	1/20 PER CENT		1/30 PER CENT		1/40 PER CENT		CONTROL
	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	
Typhi murium 0231...	8	26	few	68	few	91	234
Hog cholera 052.....	1	4	few	15	2	6	250
Danysz B.....	num.	113	num.	num.	num.	num.	151
Guinea pig Paratyph. B 34.....	0	0	0	0	0	0	159
Hog cholera Ithaca...	f. num.	130	num.	num.	f. num.	num.	274
Danysz 540.....	num.	198	num.	num.	num.	num.	231
Typhi murium 15.....	num.	176	num.	num.	num.	num.	250
Cholera suis C.....	0	3		4			238
Danysz A.....	num.	151	num.	158			290
Danysz 590.....	num.	158	num.	num.	num.	num.	214
Hog cholera 2228.....	f. num.	105	f. num.	f. num.	f. num.	96	?
Hog cholera Page 118.	0	0	0	2	0	0	257
Suipestifer 332.....	12	20			few	48	233
Typhi murium 237....	num.	102			num.	92	130
Suipestifer 553.....	num.	300			num.	num.	412
Typhi murium 0299...			0	0	0	0	158
Hog cholera 3890.....			num.	182	num.	101	352
Typhi murium 0301...	num.	102	num.	num.			147
Hog cholera 138.....	0	0	0	12			366
Suipestifer 258.....	few	40	f. num.	72	f. num.	74	339
Typhi murium 0300...	num.	240	num.	num.	num.	num.	325
Hog cholera 048.....	f. num.	110	f. num.	num.	num.	num.	180
Suipestifer 231.....	num.	192	num.	num.	num.	num.	202
Hog cholera 053.....	few	57	f. num.	84	f. num.	96	120
Typhi murium 0229...	num.	125	num.	num.	num.	num.	163
Hog cholera 051.....	0	0	9		0	0	134
Hog cholera 050.....	num.	133	num.	num.	num.	num.	124
Hog cholera 049.....	few	90	few	127	f. num.	141	219
Rabbit paratyph. B 45			3	8	2	6	365

For the sake of clearness the results of these experiments are summarized in table 3.

It is seen that all of the *B. Danysz* cultures and 5 of the 7 *B.*

TABLE 3

	NUMBER OF STRAINS SHOWING ON VICTORIA BLUE 4R. AGAR		
	Marked inhibition	Moderate inhibition	No inhibition
B. suipestifer	6	1	9
B. typhi murium	1	1	5
B. Danysz	0	0	4

typhi murium cultures showed good growth on Victoria blue agar. The hog cholera organisms fall into two sharply defined groups; 6 of the cultures are almost completely inhibited, like paratyphoid B strains, 9 strains are not inhibited and only one strain shows moderate inhibition. Jordan (1917) says:

A few strains of porcine origin possess the characteristics of the *B. paratyphosus B.* type. These, however, are all strains that have been under cultivation for some time. A number of strains, particularly some of the older cultures, have shown marked variations since they came into my hands. Some of the difficulty experienced by previous observers in the differentiation of the *B. paratyphosus* and *B. suipestifer* types has been probably due to the existence of stock cultures labelled in one way or the other, but possessing the cultural and agglutinative character of the opposite type. The extent to which transformation of one type into the other occurs under the ordinary conditions of laboratory cultivation is a matter for further investigation.

In view of this situation with regard to the *B. suipestifer* cultures, one is tempted to conclude that paratyphoid B, alone of all the paratyphoid enteritidis group of organisms is inhibited in its growth by the strength of Victoria blue 4 R. employed in the above experiments. It would seem highly desirable to repeat the experiments with freshly isolated strains.

REFERENCE

JORDAN, E. O. 1917 Jour. Inf. Dis., **20**, 477.

AUTOMATIC WATER LEVEL FOR ARNOLD STERILIZERS

IVAN C. HALL

Department of Pathology and Bacteriology, University of California, Berkeley

Experienced technicians, and untrained students, alike err occasionally in allowing Arnold sterilizers to burn dry, involving rapid depreciation of the apparatus and inconvenience incidental to the necessity of repairs.

The following arrangement has been in operation for some time in our laboratory where over one hundred and seventy-five different persons use the group of twelve connected sterilizers with absolutely no required attention on their part to the water supply.

The water main is connected to the flush tank of a water closet, placed on the bench at the end of the battery of sterilizers. The inlet valve is operated by the rise and fall of the usual brass globe, so that a constant level is maintained. In case any defect should cause the valve to fail to close, an overflow is arranged to carry the surplus water to the sewer, thus avoiding the possibility of a flooded room. This contingency has not yet arisen. The sterilizers which stand upon gas plates are connected with the supply tank and with one another by means of siphons made of $\frac{3}{8}$ inch lead pipe bent into U tubes. These are started by filling with water and holding the ends closed with the fingers until one end can be released under the water in the tank. Proceeding thus from the tank to the first sterilizer, and from this to the second, the successful operation of the connecting siphons was established to start with one at a time.

The water, of course, soon reaches a common level in all, as determined by the setting of the float valve. Any operation of the sterilizers draws upon the supply tank through the siphons for water sufficient to maintain the common level, and the lowering of the float operates the valve automatically to replenish the supply as needed.

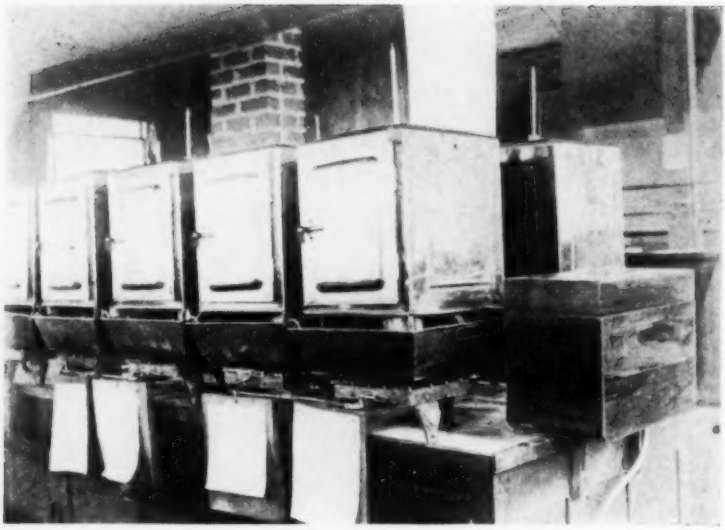


FIG. 1. AUTOMATIC WATER LEVEL FOR ARNOLD STERILIZERS, SHOWING METHOD OF ASSEMBLY WITH CONNECTING SIPHONS

AN AEROBIC SPORE-FORMING BACILLUS GIVING GAS IN LACTOSE BROTH ISOLATED IN ROUTINE WATER EXAMINATION

E. M. MEYER

Laboratory for Field Investigations of Stream Pollutions, United States Public Health Service, Cincinnati, Ohio

Received for publication May 20, 1917

In the course of routine water examinations at this station, the writer has, on several occasions, isolated aerobic lactose-fermenting organisms which have been demonstrated to be spore-forming. So far as can be ascertained by a fairly complete review of the published literature, there has been no such organism previously described.[1] In water work two large groups of bacteria generating gas from lactose are recognized. The first are the aerobic non-spore-forming bacilli of the coli-aerogenes group, and the second the anaerobic spore-forming bacilli of the sporogenes group. The significance of the presence in a water of members of either of these groups has been pretty well established. The organism to be described lies midway between these two groups, in that it is aerobic as well as spore-forming. Just what its sanitary significance is, remains to be established.

ISOLATION

It has been the routine procedure in this laboratory to isolate for study one culture of *B. coli* from each sample of water examined. This is effected by carefully fishing one isolated colony

[1] Since this article went to press, the writer has come across an article on systematic bacteriology of water by S. De M. Gage and E. B. Phelps in Am. Pub. Health Ass. Rep. 1902, **28**, 402, 412. In the table given in this article a few cultures are noted as being aerobic lactose fermenters. In the opinion of Mr. Gage it is very unlikely that the organism now being described could be among those described by him.

from the confirmatory Endo's medium plate, made from the lactose broth tube inoculated with the water sample. The colony is inoculated into lactose broth, to confirm gas production, and if this tube is positive the organism is again plated on Endo's medium to determine if it be pure. If this plate reveals the presence of but one form, a colony is fished to an agar slant. A smear from the culture on the slant, after forty-eight hours' growth at 37°C., is stained by Gram's method, and if only Gram-negative, non-spore-forming bacilli are seen, the culture is considered pure and made up of *B. coli*.

Following the above outlined procedure, eight cultures from one source[2] were found which gave gas in lactose broth, but which on the agar slant grew very differently from *B. coli*. They appeared on staining to be large Gram-negative, fusiform rods containing spores, together with large Gram-negative vegetative bacilli. For some time the writer was under the impression, gained through previous experience, that this spore-bearer was a contamination form and that the gas production would finally be found to be due to *B. coli*. Accordingly, attempts were made to purify the culture by plating on Endo's medium, agar and gelatine. The colonies on all of the media appeared to contain but one organism. When transferred to agar slants the growth was macroscopically and microscopically like the agar slant culture first alluded to. That the gas production was due to the spore-forming organism was definitely proved by the heat-resistance experiments next performed.

RESISTANCE TO HEAT

The organism will live and generate gas in lactose broth after being subjected to 95°C. for twenty minutes or to boiling water temperature for ten minutes, but is killed if subjected to the latter temperature for fifteen minutes. The writer has been checked in these results by another bacteriologist in this labora-

[2] Since the writing of this article the organism has been isolated three additional times—once from the tap water of Covington, Ky., and twice from raw tannery trade wastes. These three cultures resemble the eight previously isolated in all particulars.

tory, working independently. The tests were run as follows: tubes of standard lactose broth, with inverted vials, were placed in a water-bath, brought to the desired temperature and regulated to within 1°C. After the tubes had attained the temperature of the water-bath, 0.1 cc. of a light suspension of the organism taken from the surface of a seventy-two hour old agar slant was carefully added to the tube and the time noted. At suitable intervals, two tubes were withdrawn and put into cold water to cool. After seventy-two hours' incubation at 37°C. the tubes were examined and the growth and gas production recorded. Where there was no gas production the tubes were sterile. Agar slant cultures made from the broth tubes showing gas revealed the spore-former in pure culture.

MORPHOLOGY AND STAINING CHARACTERISTICS

In smear preparations made from forty-eight hour old agar slants three distinct forms, representing different stages of the bacillus, are seen. First there is the vegetative cell, a regular rod with rounded ends, Gram negative, 4.5 to 5.5 by 0.8 to 1 μ Next is the spore-bearer, spindle-shaped, Gram-negative, about 3 by 1.5 μ. With ordinary stains the spore is shown as a central, oval, unstained area, and with Müller's spore stain is readily demonstrated. The third form is the free spore, oval, regular, 2 by 1.5 μ, showing deep red with the spore stain. These occur most frequently and regularly in older cultures.

No capsules could be demonstrated by the use of the Welch capsule stain in smear preparations from milk cultures. When stained by Löffler's method, the organisms show numerous peritrichic flagella, some bacilli showing as many as 16 to 18. Notwithstanding this fact, motility has not been observed when using the hanging drop method.

APPEARANCE AND REACTIONS IN VARIOUS MEDIA

Agar slant. Growth quite distinctive. At 37°C., in twenty-four hours, thin transparent veil-like growth over entire surface except the very top. Growth lobate along upper edge. Micro-

scopically—in twenty-four hours mainly vegetative forms, in forty-eight hours spore-bearing forms and later only free spores.

Agar stab at 37°C. Growth along entire line of inoculation into depths of agar. Growth somewhat echinulate. In meat infusion agar many gas bubbles, due to fermentation of muscle sugar (inosite).

Agar plate. Nutrient agar twenty-four hours at 37°C., colonies discrete, round, thin and small (1 mm. diameter), edges smooth.

Endo's plates at 37°C. Twenty-four hours, colonies pink with red center, irregular contour, 1 to 2 mm. diameter, little or no sheen. Colonies forty-eight hours, deep red, much sheen in colonies and surrounding medium. Latter point distinctive.

Gelatin plate at 20°C. Forty-eight hours, colonies small (0.5 to 1 mm. diameter), round, *incipient* liquefaction. Colonies seventy-two hours—liquefaction, round, edges regular, 2 to 3 mm. diameter.

Gelatin stab at 20°C. Forty-eight hours, beginning liquefaction; in seventy-two hours liquefaction infundibuliform, slight precipitate.

Carbohydrates. In standard extract broth to which has been added one percent of the following carbohydrates, acid and gas are formed: (1) Glucose, (2) laevulose, (3) raffinose, (4) maltose, (5) sucrose, (6) lactose, (7) inulin, (8) starch, (9) glycerol, (10) mannitol. No acid or gas and little growth in dulcite broth, which remained clear and limpid. In other broths gas usually appeared in twenty-four hours. Media uniformly clouded, slight stringy precipitate, no pellicle. Media forty-eight hours, slightly viscous.

Glucose neutral-red broth. Same reaction as that of *B. coli*, i.e., yellow fluorescence with gas formation.

Clark's glucose, peptone, phosphate, medium (0.5 per cent glucose, 0.5 per cent K_2HPO_4 and 0.5 per cent peptone broth), typical reaction of "Grain" type coli, in forty-eight hours at 37°C., i.e. reaction alkaline to methyl red, Voges-Proskauer test positive.

Limiting hydrogen-iron concentrations. Clark's glucose peptone-phosphate medium was used after being adjusted with NaOH or HCl to various hydrogen-ion concentrations. Growth between $P_H = 5.0$ to $P_H = 9.0$ inclusive. In tube $P_H = 9.0$ very scanty growth and only bubble of gas. In all other tubes much gas, and growth luxuriant.

Indol production in 1 per cent peptone, four days at 37°C. No indol detected when tested for by the nitrite and by Ehrlich's para-dimethyl-amido-benzaldehyde method.

Reduction of nitrates. In 0.1 per cent peptone + 0.02 per cent $NaNO_2$ solution, four days at 37°C., no reduction to nitrites.

Litmus milk at 37°C. In twenty-four hours acid, in forty-eight hours partially reduced, coagulated with extrusion of whey, beginning digestion of curd.

Lactose bile at 37°C. Ninety-six hours, no gas or growth.

Chromogenesis. None noted on any media used.

OCCURRENCE AND SIGNIFICANCE

Dr. J. S. Bolten, working in this laboratory, isolated what the writer believes to have been the same or a similar organism from a sample of sewage which had been taken from Mill Creek during the winter of 1916 and stored for forty-one days. Reference to his unpublished notes which are on file at this station, shows that the organism had morphological and cultural characteristics similar to those detailed above. Owing to rather variable and uncertain heat resistance experiments, which Dr. Bolten deemed inconclusive, he was unable to state definitely that the spore-bearer was the gas-former. However, Dr. Bolten used for seeding material in the heat resistance experiments, cultures in lactose broth, in which medium, sporulation is indefinite and delayed.

Regular examinations are made in this laboratory of samples of water from various sources. These include the Ohio River and its tributaries in the vicinity of Cincinnati, and the tap supplies of Cincinnati and two Kentucky cities on the Ohio River opposite this city. The organism herein described has

been isolated from but one of these sources—the water supply of Newport, Kentucky. Up to date it has been obtained from the samples collected on the following eight days: January 30, February 8, 10 and 26, March 31, and April 2, 11, and 20, 1917. Newport, Kentucky, uses Ohio River water after subjecting it to treatment and storage. Treatment consists of addition of small amounts of calcium hypochlorite, and of lime and iron, and the total storage is estimated at twenty days.

This organism during the times of its occurrence might cause considerable error in the determination of the colon index. During the months of January to April, 1917, inclusive, of 40 aerobic gas formers isolated from 91 samples of Newport tap water, 32 or 80 per cent were of the coli-aerogenes group while 8 or 20 per cent were this spore-former. Due to its rarity and limited occurrence, however, it could not constitute a source of appreciable error in routine water examinations in most localities. Over 17,000 samples have been examined at this station within the past three years, and this spore-former has been isolated but eight times and, as detailed above, from but one source. Other water workers have never reported its occurrence, and it is likely that in most waters it is exceedingly rare, if not entirely absent.

SUMMARY

An aerobic bacillus, giving gas from lactose, and demonstrated to be spore-forming, has been isolated eight times between January 30 and April 20, 1917, from the tap water of Newport, Kentucky. This organism is believed to be a species whose isolation has never before been described.

The writer wishes to acknowledge his indebtedness to Surgeon W. H. Frost, under whose direction this study has been made.

A SIMPLE METHOD FOR THE CLASSIFICATION OF BACTERIA AS TO DIASTASE PRODUCTION

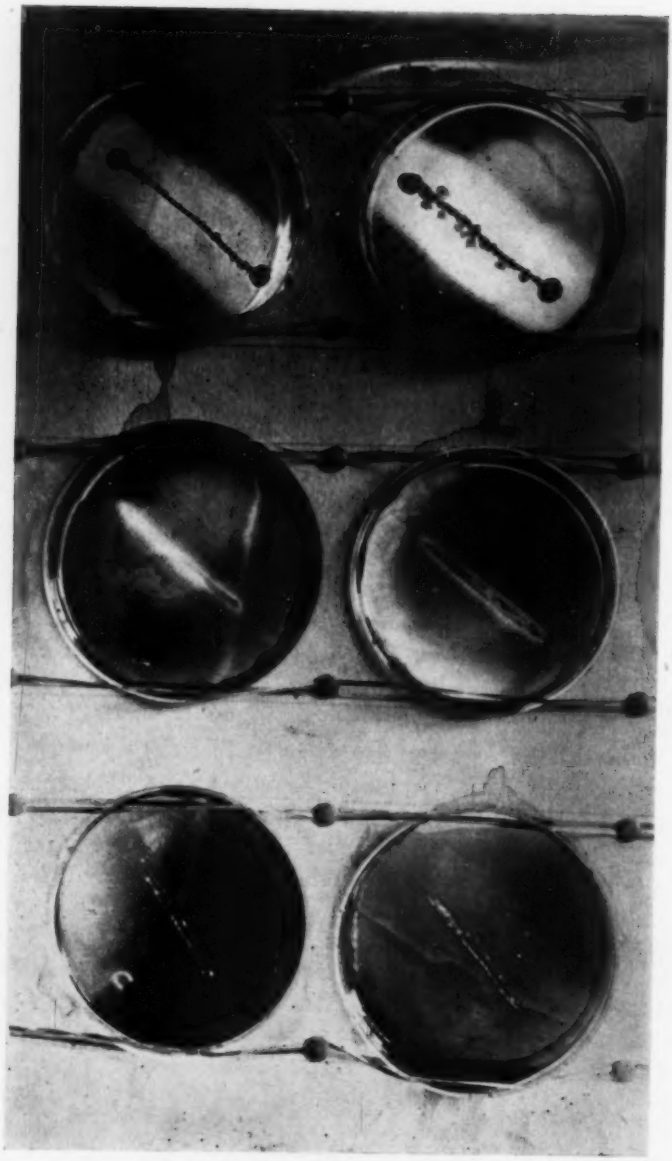
PAUL W. ALLEN

Dairy Bacteriology Laboratory, University of Illinois

Received for Publication May 25, 1917

Among the various physiological reactions selected by the Classification Committee of the Society of American Bacteriologists for determining the identity of an organism the diastasic action presents the greatest difficulties. In all tests (seven are represented in the group number of the Society's classification card) of the activity of bacteria toward certain substances the aim is accuracy and ease of application. As at present carried out, the diastase test is far from satisfactory, not only because of the difficulty in obtaining potatoes of uniform quality, but also because of the difficulty arising when one tries to make a sharp distinction between "strong" and "feeble action." If the action is vigorous or if it is very feeble it is easily classed, but there are points between these extremes which produce doubt and the test then becomes a matter of mere judgment.

During the last two years in the Dairy Bacteriology Laboratory of the University of Illinois the use of potato slants for the determination of diastase production by bacteria has been replaced by a simple plate method as follows: A starch agar is made by adding 0.2 per cent of water-soluble starch to the regular plain agar. This starch agar can be sterilized in the autoclave along with other media. Some hydrolysis takes place, but not enough to interfere with the test. The agar is poured into Petri dishes and allowed to cool, when a stroke 2 inches long is made with a loopful of an agar slant growth of the organism to be tested. The plates are incubated for two days at 37°C. and five days at 20°C., after which they are flooded with a saturated solution of iodine in 50 per cent alcohol.



DIASTATIC ACTION "STRONG," "FEEBLE," AND "ABSENT"

A clear area about the stroke averaging more than 2 mm. in width classifies the diastatic action as *strong*, while a width of 2 mm. or less marks it as *feeble* and the absence of a halo as *absent*. These are the three terms used on the classification card of the Society of American Bacteriologists. As the loopful of growth from an agar slant is the unit amount of inoculation which is used in all of the other tests which go to make up the group number of an organism, the starch agar test is consistent in this respect.

As used in this laboratory, some of the evident advantages of this method over the potato slant method are the decrease in time and labor in the preparation of the media, and the greater accuracy which the average worker can obtain.

The starch agar gives best results if used before it is two weeks old, as after that period of time the starch becomes changed and spots of reddish purple develop on the addition of iodine solution.

As to the comparison of this method with the potato test, it has been found that for the organisms of dairy products, approximately the same results are arrived at whether one test or the other is used.

A SIMPLE AND PRACTICAL MEDIUM FOR DIFFERENTIATING B. TYPHOSUS, B. PARATYPHOSUS A., B. PARATYPHOSUS B., B. ENTERITIDIS, AND B. COLI

KAN-ICHIRO MORISHIMA

From the Quarantine Laboratory, Port of New York, Health Officer's Department, Rosebank, New York

Received for publication May 14, 1917

Before the war in Europe began paratyphoid fever was relatively rare, but owing partly to prophylactic inoculation against typhoid, it soon became more common among soldiers than the latter disease. With increased prevalence there is need of simple cultural tests for the recognition of the causative organisms. A number of such tests have been devised. Russell's double sugar medium, consisting of litmus agar with 1 per cent lactose and 0.1 per cent glucose, is widely used to receive the fishings of suspicious colonies. It differentiates the paratyphoids and *B. enteritidis* from *B. typhosus* and the *B. coli* group, but does not differentiate between *B. paratyphosus A*, *B. paratyphosus B.*, and *B. enteritidis*. To determine which of these latter organisms is present, further inoculation of the culture into litmus milk, lead acetate agar, or other special culture media, and agglutination tests must be resorted to.

It occurred to me that by pouring one agar medium into a test tube and allowing the agar to solidify, and then pouring a layer of a second differential agar medium upon this, a medium would be obtained which would furnish, in twenty four hours, more information from a stab fishing than do the media at present employed. Of a large number of such combinations, the following proved to be the most satisfactory.

Meat infusion agar is prepared in the usual way, cleared with egg-white, and titrated while hot to about -0.2 to -0.4 with phenolphthalein as an indicator. A 2 per cent solution of neu-

tral lead acetate in sterile distilled water is prepared and heated for one-half hour at 100°C. in a water bath. Five cubic centimeters of the solution is added to 100 cc. of the agar, melted and cooled to about 60°C. This lead acetate agar is transferred to small test tubes to a depth of about 1.5 cc. If the agar with the lead acetate is tubed while hot, a precipitate containing most of the lead settles to the bottom of the tube; this may be prevented by cooling the agar to 60° or 70°C. before tubing it.

A 1 per cent stock solution of china blue in distilled water is kept on hand. Four-tenths of a cubic centimeter of normal sodium hydrate are added to 10 cc. of the china blue solution, and the mixture is heated on the water bath for ten minutes at 100°C. The color changes from blue to brown during the heating. One and one-fifth cubic centimeters of this decolorized china blue solution is added to 100 cc. of nutrient agar of reaction, −0.2 to −0.4. One per cent lactose and 0.1 per cent glucose are added and the mixture is heated ten minutes in the water bath at 100°C. The medium is cooled to about 60°C, and is then ready to be added as a second layer to the small tubes containing the lead acetate agar. It forms a layer of about the same depth as the latter medium. The tubes are incubated overnight, and any contaminated ones are discarded.

The results of stab cultures in this medium after ten to eighteen hours' incubation may be summarized as follows:

CULTURE	GAS PRODUCTION	BOTTOM LAYER	TOP LAYER
B. typhosus	−	Black	Pale blue
B. paratyphosus A	+	No change	Pale blue
B. paratyphosus B	+	Black	Colorless
B. enteritidis	+	Black	Colorless
B. coli	++	Black, or no change	Deep blue

It is thus seen at a glance on the morning after the suspicious colony is fished, whether the organism is *B. paratyphosus A* or *B*, the typhoid bacillus, or *B. coli*. The medium does not differentiate between *B. paratyphosus B* and *B. enteritidis*. By in-

creasing the amount of the upper layer and slanting it, a streak culture can be made before stabbing, as in the case of the Russell medium, and material will thus be available for agglutination tests without further transplantation. The medium is readily prepared, and is inexpensive. It has been preserved for one month in the ice-box without deterioration; by adding two drops of sterile liquid paraffine to prevent evaporation, it can probably be kept indefinitely.

I next attempted to prepare a medium that would differentiate between *B. paratyphosus B* and *B. enteritidis*, as well as between these organisms and *B. paratyphosus A*, *B. typhosus*, and *B coli*. The following represents the best combination obtained for this purpose. Sugar-free agar is used for both the lead acetate and the china blue; 1 per cent lactose, 1 per cent inosite, and 0.1 per cent arabinose are added to the china blue agar. With this medium the lead acetate layer behaves toward the different organisms as in the preceding medium, and all of the organisms produce gas in it, except *B. typhosus*. The shade of blue produced in the upper layer after twenty-four hours' incubation may be indicated as follows:

B. typhosus	+	
B. paratyphosus A	+	
B. paratyphosus B. sub. 1	+ +	
B. paratyphosus B. sub. 2	−	or trace
B. enteritidis	−	or trace
B. coli	+ + +	

B. paratyphosus B has been divided into two groups by Weiss, according to whether the organism does or does not ferment inosite; most of the strains ferment inosite, and so fall into his sub 1 group. Therefore, the medium just described differentiates sharply between most paratyphoid B strains and *B. enteritidis* but not between paratyphoid B sub 2 and *B. enteritidis*. Fourteen of our paratyphoid B. strains belong to the sub 1 group, and only five to the sub 2 group. The medium differentiates quite sharply,—*B. enteritidis* and *B. paratyphosus* sub 2 remaining colorless or producing a mere trace of blue; *B. typhosus* and *B. paratyphosus B* showing pale blue; *B. paratyphosus B*

sub 1 becoming several shades deeper blue, and, finally, *B. coli* showing a dark blue.

The inosite used in these experiments was kindly furnished by Dr. W. G. Lyle. I am indebted to Dr. Oscar Teague for suggestions during the progress of this work.

A NEW APPARATUS FOR OBTAINING SIMULTANEOUS CULTURES OF ANY DESIRED AGE FOR COMPARATIVE STUDY

R. G. PERKINS

From the Laboratories of Hygiene and Bacteriology, Western Reserve Medical School

Received for publication June 2, 1917

In the course of a series of investigations of the morphology of *B. diphtheriae*, it became necessary to study cultures of different ages on account of the well recognized fact that the morphology varies from hour to hour. It is of course simple enough to get eighteen-hour cultures, twenty-four-hour cultures and cultures five, six or seven hours old, but unless the investigator is living at or near the laboratory, there are many intervals which are very inconvenient. To avoid this difficulty, and to avoid also the multiplicity of inoculations necessary for any long series, an attempt was made to develop an apparatus which would give one a complete series of cultures of different ages with a single inoculation. This has been done successfully in the following manner.

Through the Waterbury Clock Company a small eight-day movement was obtained which had been so arranged that the hour hand revolved once in twenty-four hours instead of once in twelve hours, as do the twenty-four-hour clocks much used by foreign railroads. This was set horizontally and a table arranged to be carried by the twenty-four-hour wheel. The table was so fitted that vertical swab holders could be fastened at various points and would of course make a twenty-four-hour circle. On a vertically adjustable hanger an open inverted plate containing a solid medium was suspended so that the surface of the agar was parallel to and immediately above the swab-carrying table. When the sterile swab was inoculated with a given culture and by means of the adjustment brought into contact with the under

surface of the plate, and the clock started, a complete-circuit smear was made covering all time periods from nothing to twenty-four hours. By marking the bottom of the plate the exact age of any given point could readily be determined. The apparatus, as illustrated in the accompanying photographs, which are self-explanatory, has worked successfully, but requires certain precautions for its use. In the first place, unless the apparatus is contained in a practically saturated atmosphere, the plate will dry out. We therefore cover the machine with a bell jar set in a water seal at the base. In the earlier work a cotton swab inoculated with the culture was used, but a theoretical objection to this method was the possibility of growth in the swab, which might interfere with the accurate morphological time interpretations. Even so, it would be at least as accurate as the method of direct inoculations, which places organisms of all ages on the inoculated media. To obviate this possible criticism, sterile water with a suspension of the organisms was used, but this apparently took up sufficient nutriment from the culture medium to have a similar result, as indicated by a heavier growth in the later periods. We now use small flaps of sterile tinfoil about 0.5 cm. in width, and find that the capillary attraction of the surface of the medium gives a good continuous contact. By this means organisms are left at the margin of the sliding foil and develop under uniform conditions at all parts of the smear. Organisms, for instance, which have left the inoculating foil at a point half way round the plate are twelve hours old, and so on.

It is obvious that for satisfactory results the medium must be of a proper consistency, and that its surface and that of the swab-carrying table must be parallel. With these precautions it is easy to obtain an even smear with separate colonies along the track of the swab. At the end of the twenty-four hours one can remove the plate, and by the use of a series of sterile cover slips get impressions of the entire circle, which may readily be labeled so that any given hour of growth can be studied with considerable accuracy. If it is desired to study cultures twenty-four to forty-eight hours old, the plate can be removed from the

apparatus, covered, and returned to the incubator for twenty-four hours. The cultures first recorded as twelve hours will then be thirty-six hours old, and so on.

The apparatus appears to have a variety of other possible uses, inasmuch as it is easy to set up relay make-and-break connections, so that an incubator or a heating apparatus of any sort may be turned on and off as many times as desirable up to twenty-four hours instead of twelve, which is obviously a limit of insufficient extent. It is felt that applications of this character may be of a good deal of value in the study of conditions involving time comparisons, and it is hoped that others may be able to find the scheme of assistance in their work. The original apparatus was constructed by the writer out of scrap materials at a total expense, including the clock ($5.50) and exclusive of time, of about $6. The improved apparatus was cast in aluminum and built up by the laboratory technical assistant at an expense, exclusive of labor and inclusive of the clock, of about $11. Details of model and construction will gladly be furnished to those interested.

DESCRIPTION OF PLATES 1 AND 2

The numbers on both plates refer to the same parts.

1, Main frame containing clock and with gutter for water seal. The number in the detailed print lies in the gutter.

2, Cover over clock with openings for shafts and regulator.

3, Revolving table turning once in twenty-four hours.

4, Clamp for Petri plate, with vertical and horizontal adjustments to fit any plate from 2 to 4 inches in diameter.

5, Vertical shaft with rack to fit pinion adjustment under No. 4.

NOTE.—This rack and pinion was taken from an old microscope. The openings in No. 2 should be covered during use by an oiled felt washer to keep the steel parts of the clock from rusting in the saturated atmosphere.

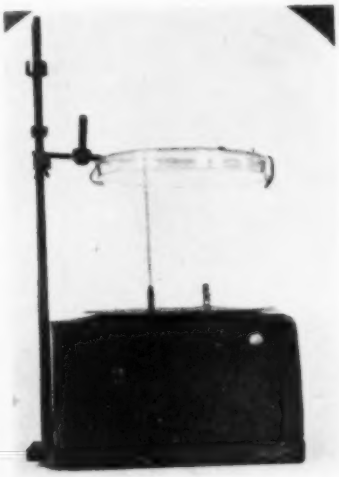


FIG. 1. ORIGINAL MODEL

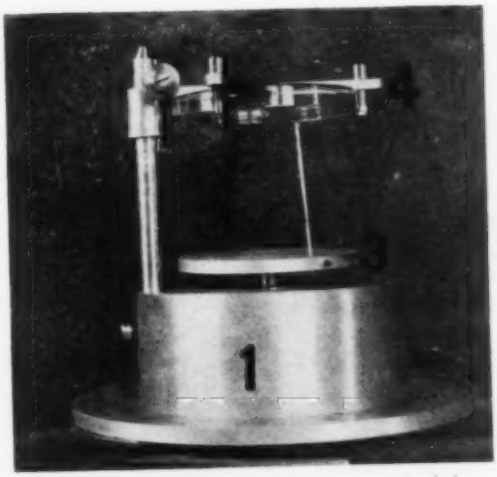


FIG. 2. PRESENT MODEL

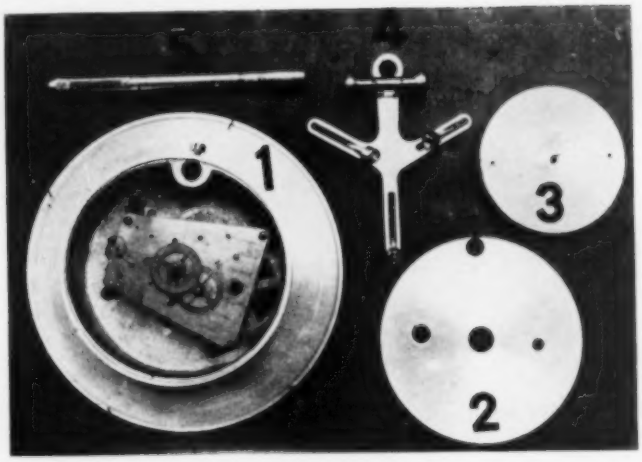


FIG. 3. DETAILED PRESENT MODEL

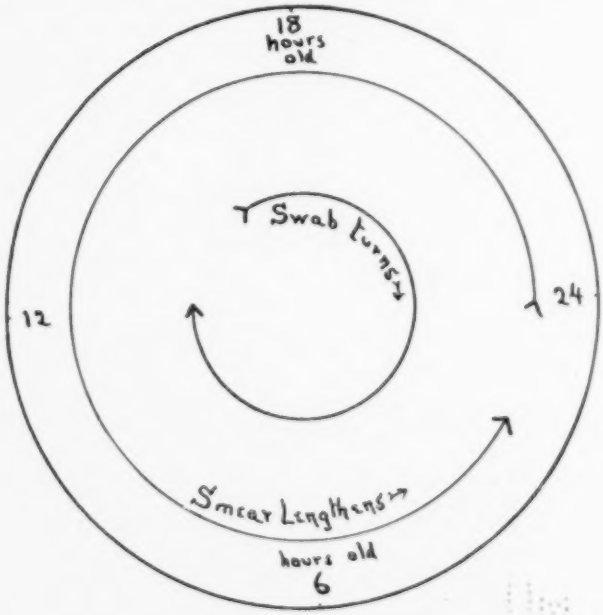


FIG. 4. WORKING DIAGRAM

(Perkins: Obtaining Simultaneous Cultures)

STUDIES IN THE NOMENCLATURE AND CLASSIFICATION OF THE BACTERIA

V. SUBGROUPS AND GENERA OF THE BACTERIACEAE

R. E. BUCHANAN

From the Bacteriological Laboratories of Iowa State College, Ames, Iowa

Received for publication October 22, 1916

Family II. Bacteriaceae Cohn, 1872a, p. 237

Synonyms:

Bacterina Perty, 1852, p. 159
Microbacteria Cohn, 1872, p. 167
Desmobacteria Cohn, 1872, p. 173
Bacteriaceen Zopf, 1883, p. 45
Baculogenae Trevisan, 1885, p. 92
Bacteriacei Schroeter, 1886, p. 155
Bakteriaceen Hueppe, 1886, p. 142
Arthrobakteriaceen Hueppe, 1886, p. 149
Bacteriacees Cornil and Babes, 1890, p. 151
Bacillaceae Fischer, 1895, p. 139
Bacillacei Fischer, 1895, p. 139.

Cells rod-shaped, straight, or at least not spherical or spiral. Never containing sulphur granules, nor with bacterio-purpurin, many species pigmented. May or may not be motile by polar or diffuse flagella. Cells may be single or in chains. Endospores produced in some genera, not in others. A pseudoplasmodium never developed. Growth energy not secured by the oxidation of ammonia or nitrites.

The following names have been used for subdivisions of *Bacteriaceae* as here defined:

Bacterieae Trevisan, 1879, p. 136
Eubacterieae Trevisan, 1879, p. 136

Bacilleae Trevisan, 1889, p. 12.
Eubacilleae Trevisan, 1889, p. 939
Klebsielleae Trevisan, 1889, p. 1028
Kurthieae Trevisan, 1889, p. 929
Pasteurieae Trevisan, 1889, p. 12
Bactrillei Fischer, 1895, p. 139
Bactrinei Fischer, 1895, p. 139
Bacillei Fischer, 1895, p. 139
Mycobacteriaceae Chester, 1897, p. 63
Clostridieae Fischer, 1903, p. 60
Plectridieae Fischer, 1903, p. 61
Acidobacteriaceae Jensen, 1909a, p. 344
Alkalibacteriaceae Jensen, 1909a, p. 343
Butyribacteriaceae Jensen, 1909a, p. 343
Luminobacteriaceae Jensen, 1909a, p. 303
Putribacteriaceae Jensen, 1909a, p. 343
Oxydobacteriaceae Jensen, 1909a, p. 343

De Toni and Trevisan (1889, p. 939) separated the various tribes and subtribes of their subfamily, *Baculogenae* in the following manner:

Key to Tribes and Subtribes of Baculogenae. DeToni and Trevisan

A. Bacilli and cocci naked, never with a capsule.....Tribe I. *Bacilleae*
I. Producing endospores.
1. Division longitudinal.....Subtribe 1. *Pasteurieae*
2. Division transverse.
a. Rods united into a reticulate coenobium
Subtribe 2. *Thiodictyeae*
b. Rods not united into a reticulate coenobium.
(1) Rods never spiral.....Subtribe 3. *Eubacilleae*
(2) Rods spirally bent.....Subtribe 4. *Spirilleae*
II. Producing arthrospores.....Subtribe 5. *Pacinieae*
B. Bacilli and cocci surrounded by a special membranous or gelatinous capsule.....Tribe II. *Klebsielleae*
I. Rods straight or curved, never true spirals.
Subtribe 1. *Euklebsielleae*
II. Rods spirally twisted.....Subtribe 2. *Myconostoceae*

Of these names *Pasteurieae* may be discarded because *Pasteuria* is probably a protozoan. *Thiodictyeae* includes forms

belonging to *Thiobacteria; Spirilleae, Pacineae,* and *Myconostoceae* are based upon genera now placed with the *Spirillaceae.*

Fischer (1895, p. 139) subdivided his family *Bacillacei* as follows:

Key to subfamilies of Bacillacei. Fischer

A. Non motile. Non flagellate.....I. *Bacillei*
B. Motile. With flagella
  I. Polar flagella only.
    a. A single polar flagellum.....II. *Bactriniei*
    b. A tuft of polar flagella.....III. *Bactrillei*
  II. With diffuse flagella.....IV. *Bactridiei*

Chester (1897, p. 63) grouped certain genera to constitute the family *Mycobacteriaceae.* The most important differential characters are the formation of clavate-cuneate cells, lack of endospores, and true dichotomous branching.

Later Fischer (1903, p. 60) separated his family *Bacillaceae* into three subfamilies as follows:

Key to subfamilies of Bacillaceae. Fischer

A. Spore-bearing rods unchanged in shape, cylindrical. Includes all non-sporulating forms.....Subfamily I. *Bacilleae*
B. Spore-bearing rods modified in shape.
  I. Spore-bearing rods spindle shaped.....Subfamily II. *Clostridieae*
  II. Spore-bearing rods drumstick shaped..Subfamily III. *Plectridieae*

The families proposed by Jensen (1909a, p. 344) which would be included under our definition of *Bacteriaceae* may be separated by the following key:

Key to the families of Bacteriaceae. Fischer

A. Without spores, motile or non-motile; when the former, with polar flagella, typically water forms, securing energy almost exclusively by oxidative processes.
  I. Obligate aërobes, oxidising carbon, hydrogen or nitrogen compounds without production of noteworthy amounts of unoxidized split products.....I. *Ozydobacteriaceae*
  II. Luminous and fluorescent bacteria.....II. *Luminobacteriaceae*
B. With or without spores, either peritrichous or non-motile, not typically water forms, do not secure energy exclusively as "A."
  I. Typically not obligate anaërobic or micro-aërophilic.
    a. Usually producing acids from carbohydrates.
      I. *Acidobacteriaceae*
    b. Usually bringing about an alkaline reaction by the development of ammonia.....II. *Alkalibacteriaceae*

II. Typically anaërobic or micro-aërophilic.
a. Characteristically producing butyric acid from carbohydrates.....I. *Butyribacteriaceae*
b. Characteristically bringing about putrefactive changes in proteins.....II. *Putribacteriaceae*

An examination of the genera which have been proposed by various authors would seem to indicate that an important differential character is that of endospore production. Separation upon this basis throws all the spore-bearing rods together, a grouping which appears to be wholly natural and to represent true relationships. Of the rod-shaped organisms which do not produce spores it seems that the acid-fast bacteria related to the tubercle bacillus likewise represent a very distinct group.

For the three tribes of the *Bacteriaceae* the names *Bacilleae Bacterieae*, and *Mycobacterieae* may be used. The principal differential characters may be summarized in the following key:

Key to the Tribes of the Bacteriaceae

A. Cells not acid-fast.
I. Typically producing endospores under favorable conditions. Tribe I. *Bacilleae*
II. Not producing endospores.....Tribe II. *Bacterieae*
B. Cells acid-fast, frequently showing some tendency to branching Tribe III. *Mycobacterieae*

Tribe I. **Bacilleae** Trevisan 1889, p. 12 in part

Synonyms:
Eubacilleae Trevisan, 1889, p. 939 in part
Bactrillei Fischer, 1895, p. 139 in part
Bacillei Fischer, 1895, p. 139 in part
Bactrinei Fischer, 1895, p. 139 in part
Bacilleae Fischer, 1903, p. 60 in part
Clostrideae Fischer, 1903, p. 60 in part
Plectrideae Fischer, 1903, p. 61 in part
Alkalibacteriaceae Jensen, 1909a, p. 343 in part
Butyribacteriaceae Jensen, 1909a, p. 343 in part
Putribacteriaceae Jensen, 1909a, p. 343 in part

Cells rodshaped, never spiral or strictly filamentous, single or in

chains, usually motile by means of peritrichous flagella, producing endospores under suitable conditions of growth, usually Gram-positive.

The following pseudogeneric names of organisms within this tribe have been employed, but never have been used with a species or in a strict generic sense, and are not valid as genera.

Amylobacter Trecul, 1865, p. 432
Urobacter Trecul, 1865, p. 432
Urocephalum Trecul, 1865, p. 432
Megabacterium Billroth, 1874, p. 16
Mesobacterien Billroth, 1874, p. 16
Petalobacteria Billroth, 1874, p. 16
Streptobacteria Billroth, 1874, p. 18
Microbacterien Billroth, 1874, p. 18
Microbacteria Billroth, 1874, p. 18
Aethylbacillus Fitz, 1878, p. 48
Polybacteria Van Tieghem, 1884
Streptobacterium Billet, 1890, p. 23
Streptobacillus Preisz? Migula, 1900, p. 374
Bacterius Kendall, 1902, p. 484
Bactrillus Kendall, 1902, p. 484
Bactrinius Kendall, 1902, p. 484
Microbacterium (Billroth) Smith, 1905, p. 174
Plennobakterium Gonnermann, 1907, p. 887

The following generic names have been used, but the species are not identifiable with any degree of certainty:

Bactrella Morren, 1830, p. 1
Sporonema Perty, 1852, p. 160

Subgeneric names:

Streptobacter Schroeter, 1886, p. 158
Eu-Cornilia Trevisan, 1889, p. 998
Pleurospora Trevisan, 1889, p. 1002

The following generic name is invalid because earlier used as the generic name of a distinct group of plants.

Bactridium Fischer, 1895, p. 139
not *Bactridium* Kunze
not *Bactridium* Salisb.

Generic names which are not invalid for any of the preceding reasons are the following:

Serratia Bizio, 1823, p. 288
Bacterium Ehrenberg, 1828
Bacteridium Davaine, 1868, p. 21
Bacillus Cohn, 1872b, p. 174
Urobacillus Miquel, 1879, p. 517
Clostridium Prazmowski, 1880, p. 23
Pollendera Trevisan, 1884, p. 943
Zopfiella Trevisan, 1885, p. 93
Cornilia Trevisan, 1889, p. 21
Eubacillus Dangeard, 1891, p. 151
Granulobacter Beijerinck, 1894, p. 3
Bactrillum Fischer, 1895, p. 139
Bactrinium Fischer, 1895, p. 139
Clostrillum Fischer, 1895, p. 139
Clostrinium Fischer, 1895, p. 139
Diplectridium Fischer, 1895, p. 140
Paracloster Fischer, 1895, p. 141
Paraplectrum Fischer, 1895, p. 141
Plectridium Fischer, 1895, p. 147
Astasia Meyer, 1898, p. 49
Fenobacter Beijerinck, 1900, p. 200
Aplanobacter E. F. Smith, 1905, p. 171
Semiclostridium Maassen, 1905, p. 5
Myxobacillus Gonnermann, 1907, p. 877
Botulobacillus Jensen, 1909a, p. 343
Butyribacillus Jensen, 1909a, p. 343
Cellulobacillus Jensen, 1909a, p. 343
Putyribacillus Jensen, 1909a, p. 343
Pectobacillus Jensen, 1909a, p. 343
Metabacterium Chatton and Perard, 1913, p. 1232

The genus *Eubacillus* Dangeard is believed by Migula (1897,

p. 94) to include organisms which are not true bacteria. The type species, *Eubacillus multisporus*, has chlorophyll, is filamentous, and produces many endospores in a filament. Until this organism can again be identified and studied more closely its position is doubtful, and it should perhaps be excluded from present consideration.

The genera of the tribe *Bacilleae* may be differentiated by the following key:

Key to the genera of Bacilleae

A. Usually a single endospore formed within each cell.
  I. Aërobic, usually Gram-positive, as a rule liquefying gelatin, spores usually not distorting rods when formed.....Genus 1. *Bacillus*
  II. Anaërobic or microaërophilic usually.
    a. Spores produced at extreme tip of cells, forming typical drumsticks.....Genus 2. *Plectridium*
    b. Spores not produced at extreme tip of cells, at least not forming drumsticks. Cells usually somewhat swollen when spores are formed.....Genus 3. *Clostridium*

B. Usually a number of spores develop within a swollen cell.
Genus 4. *Metabacterium*

Genus 1. **Bacillus** Cohn, 1872b, p. 174

Synonyms:

Bactrella? Morren, 1830, p. 354
Metallacter? Perty, 1852, p. 180
Bacteridium Davaine, 1868, p. 21
Urobacillus Miquel, 1879, p. 517
Pollendera Trevisan, 1884, p. 943
Dispora? Kern, 1882, p. 135
Zopfiella Trevisan, 1885, p. 93
Cornilia Trevisan, 1889, p. 21 in part
Bacterium Migula, 1894, p. 237 in part
not *Bacterium* Ehrenberg, 1828
not *Bacterium* Cohn, 1872b, p. 167
Bactrinium Fischer, 1895, p. 139
Bactrillum Fischer, 1895, p. 139
Endobacterium Lehmann and Neumann, 1896, p. 103
Astasia Meyer, 1898, p. 49
Saccharobacter? Beijerinck, 1900, p. 200

Fenobacter Beijerinck, 1900, p. 200
Aplanobacter E. F. Smith, 1905, p. 171
Semiclostridium Maassen, 1905, p. 5
Myxobacillus Gonnermann, 1907, p. 877
Plennobacterium Gonnermann, 1907, p. 887
Serratia Vuillemin, 1913, p. 521
not *Serratia* Bizio 1823

Cells rod-shaped, straight or at least never spiral, motile by diffuse flagella or non-motile. Endospores produced under favorable conditions, not usually distorting the cell, usually Gram-positive. Growth usually good on laboratory media; commonly liquefying gelatin. Aërobic or facultative.

There has been much diversity in the literature as to the use of the generic name *Bacillus*. It was first used by Cohn (1872, p. 174) to include three species of rod-shaped organisms, *Bacillus subtilis*, *B. ulna*, and *B. anthracis*. He characterized the genus as including those rod-shaped organisms that grow in filaments. Later he discussed at length spore production in *B. subtilis*, and gave the first accurate description of endospores. Various authors accepting either *B. subtilis* or *B. anthracis* as the type of the genus have emphasized different characters so that there are current in literature at least six conceptions of the genus. These are as follows:

1. Bacillus. Rod-shaped organisms growing in filaments or in chains. Spore production, flagella distribution and motility not emphasized or regarded as secondary. Among the authors who have made use of this definition are Cohn (1872 and 1875), Magnin (1878), Winter (1879), Luerssen (1879), Van Tieghem (1884), Grove (1884), Flügge (1886), and Schroeter (1886).

2. Bacillus. Rod-shaped organisms producing endospores. (Some authors recognize other spore-bearing genera in addition to *Bacillus*.) The following have used this definition; De Bary (1884 and 1887), Zopf (1885), Hueppe (1885), Cornil and Babes (1885 and 1890), De Toni and Trevisan (1889), Ludwig (1892), Freudenreich (1894), Lehmann and Neumann (1896), Chester (1897), Flügge (1908), Jensen (1909), Heim (1911), Löhnis (1913).

3. Bacillus. Rods motile by means of peritrichous flagella, spores may or may not be produced. The following authors have recognized this definition; Migula (1894, 1895, 1897, 1900, 1904), Chester (1901), Kendall (1901), A. J. Smith (1902), E. F. Smith (1905), Ellis (1909), Frost (1911), and Schneider (1912).

4. Bacillus. Rods, non-motile, producing endospores. Fischer (1895), Lotsy (1907).

5. Bacillus. Any rod-shaped organism. Baumgarten (1890), Sternberg (1892), Mace (1897), Hewlett (1898), and Matzuschita (1902).

6. Bacillus. Any motile rod. Conn (1909).

Vuillemin (1913) has claimed that the name Bacillus has been so vulgarized by the bacteriologist because of the number of definitions that it should be abandoned as a generic designation. It is, of course, true that bacillus is used as a common designation of all rod-shaped bacteria, but this should not invalidate the use of this term as a generic name any more than the use of *Chrysanthemum* or *Aster* as genera by botanists is interfered with by the common names chrysanthemum and aster.

In some form or with some definition the genus *Bacillus* should be retained. The type practically always accepted is *B. subtilis*. The definition of Fischer should therefore be abandoned as including only non-motile forms. He would exclude from the genus its first described species. The original description of Cohn is scarcely sufficient, for much stress was laid upon cell grouping and length of cell and not upon other characters. The use of Migula's diagnosis, including in the genus all rods with peritrichous flagella, is the cause of great confusion. It brings into the genus such discordant types as the hay bacillus and the typhoid bacillus while it excludes the anthrax bacillus so closely related to the hay bacillus. Migula's definition should be abandoned as not based upon natural affinities. The definitions which would include all rods in the genus *Bacillus* have the merit of simplicity. When, however, organisms so diverse in characteristics as the tubercle bacillus, the typhoid bacillus, the tetanus bacillus, and the anthrax bacillus are all included in one genus the simplicity is more apparent than real. The existence

of such diverse forms has led most recent authors to divide bacteria into well marked groups. It is the opinion of the author that the larger of these groups should be recognized as genera. The term *Bacillus* should therefore be restricted, and it would seem that it should be defined more nearly in the terms of De Bary, Zopf, Hueppe, etc., who emphasized the importance of spore production as a diagnostic character.

The objection may be raised that a definition of *Bacillus* as a genus made up of endosporous rods would exclude forms which have lost the power of spore formation but are in other respects closely related. It is evidently impracticable to base generic diagnoses upon a single character. Even though an organism be a variant in one or even more characters, the other resemblances would be sufficient to include the organism in question in the correct genus. Illustrations of this fact may be taken from higher plants. The Lombardy poplar is always classified in the genus *Populus*. It never produces fruit, and it persists solely as the result of vegetative reproduction; yet the genus *Populus* is based in part upon certain fruit characters. The other characters are so evidently poplar-like, however, that we do not question the correctness of the assignment of this species to the genus *Populus*.

The genus *Bacillus* is a relatively large one, and shows some degree of differentiation among the species. It may to advantage be divided into subgenera.

The following key differentiates the subgenera on the basis of their most striking characteristics:

Key to the subgenera of Bacillus

A. Spore not barrel-shaped in longitudinal section and not star-shaped in cross section.
   I. Motile by means of peritrichous flagella..Subgenus 1. *Eu-Bacillus*
   II. Non-motile.....Subgenus 2. *Bacteridium*
B. Spore barrel-shaped in longitudinal section, longitudinal striations evident. Subgenus 3. *Astasia*

Subgenus 1. **Eu-Bacillus**

Synonyms:

Urobacillus Miquel, 1879, p. 517

Zopfiella Trevisan, 1885, p. 93

Bactrinium Fischer, 1895, p. 139
Bactrillum Fischer, 1895, p. 139
Fenobacter Beijerinck, 1900, p. 200
Myxobacillus Gonnermann, 1907, p. 877
Serratia Vuillemin, 1913, p. 521
not *Serratia* Bizio, 1823

Motile, usually by means of peritrichous flagella. Spores not barrel-shaped, without longitudinal ridges. Other characters those of the genus.

The type species of the subgenus is *Bacillus subtilis* Cohn.

Subgenus 2. **Bacteridium** Davaine, 1868, p. 21

Synonyms:
Pollendera Trevisan, 1884
Bacterium Migula, 1894, p. 237
not *Bacterium* Ehrenberg, 1828
not *Bacterium* Cohn, 1872b, p. 167
Bacillus Fischer, 1895, p. 139
not *Bacillus* Cohn, 1872, p. 174
Aplanobacter E. F. Smith, 1905, p. 171

Non-motile, without flagella. Spores not barrel-shaped, without longitudinal ridges. Other characters those of the genus.

The type species is *Bacillus* (*Bacteridium*) *anthracis* Cohn.

The name *Bacteridium*, according to E. F. Smith, is invalid because of the previous existence of the genus *Bactridium*, Kunze, 1817. Whether or not this invalidates *Bacteridium* Davaine depends upon the interpretation of Article 57 and Recommendation XXXI of the botanical code. The former states that when the difference between two names, especially two generic names lies in the termination these names are to be regarded as distinct even though differing by one letter only. The latter reads:

Many names differ by a single letter without risk of confusion. In cases where a close approach to identity is a source of error (ex. *Astrostemma* and *Asterostemma* in one and the same family, *Asclepiadaceae*)

only one, the older, of the names should be kept in accordance with Article 51, 4°."

This last article reads:

Every one should refuse to admit a name in the following cases:
4. When the group it designates embraces elements altogether incoherent, or when it becomes a permanent source of confusion or error.

It would seem that there is ample justification in the code for the recognition of *Bacteridium* Davaine.

Subgenus 3. **Astasia** Meyer, 1898, p. 49

Motile rods, spores ovoid with longitudinal striae, star-shaped in cross section.

The type species is *Bacillus* (*Astasia*) *asterospora* Meyer.

Genus 2. **Plectridium** Fischer, 1895, p. 147

Synonyms:

Paraplectrum Fischer, 1895, p. 139
Plectrinium Fischer, 1895, p. 139
Plectrillum Fischer, 1895, p. 139
Diplectridium Fischer, 1895, p. 140
Putribacillus Jensen, 1909a, p. 343 in part
Pectobacillus Jensen, 1909a, p. 343 in part

Cells rod-shaped, straight or at least never spiral, motile by diffuse flagella or non-motile. Endospores produced under favorable conditions, causing an enlargement of one tip of the cell, giving rise to a drumstick appearance. Usually Gram-positive. Anaërobic or microaërophilic.

The type species is *Plectridium tetani* (Nicolaier) Fischer.

Genus 3. **Clostridium** Prazmowski, 1880, p. 23

Synonyms:

Amylobacter Trecul, 1865, p. 435
Cornilia Trevisan, 1889, in part

Granulobacter Beijerinck, 1893, p. 7
Clostrillum Fischer, 1895, p. 139
Clostrinium Fischer, 1895, p. 139
Paracloster Fischer, 1895, p. 140
Semiclostridium Maassen, 1905, p. 5
Botulobacillus Jensen, 1909a, p. 343
Butyribacillus Jensen, 1909a, p. 342
Cellulobacillus Jensen, 1909a, p. 343
Putribacillus Jensen, 1909a, p. 343 in part

Cells rod-shaped, straight or at least never spiral. Frequently showing granules. Endospores usually produced in cells showing some enlargement; usually the cells become spindle-shaped. Anaërobic or microaërophilic. Usually Gram-positive.

The type species is *Clostridium butyricum* Prazmowski.

Genus 4. **Metabacterium** Chatton and Perard, 1913, p. 1232

Rod-shaped, known only in the sporulating state, from the caecum of a guinea pig. Sporogenous cell becomes ellipsoidal with one to eight endospores within a single cell.

The type species is *Metabacterium polyspora* Chatton and Perard.

The organism has not been cultivated. The diagnosis makes it evident that the organism is scarcely sufficiently known to make accurate diagnosis possible. It has been urged by Vuillemin (1913) as a *genus conservandum*.

Tribe II. **Bacterieae** Trevisan, 1879, p. 136 emended

Synonyms:
Klebsielleae Trevisan, 1889, p. 1028
Acidobacteriaceae Jensen, 1909a, p. 303 in part
Luminibacteriaceae Jensen, 1909a, p. 344

Cells rod-shaped, never spiral nor strictly filamentous; single or in chains, motile or non-motile, never producing endospores, either Gram-positive or -negative.

The following pseudogeneric names or words not used strictly in a generic sense have been applied to organisms of this subfamily.

Mycothrix Itzigsohn, 1867
Microsporon Klebs, 1871
Ascobacteria Billroth, 1874, p. [illegible]
Coccobacteria Billroth, 1874, p. 1
Diplobacteria Billroth, 1874, p. 16
Gliabacteria Billroth, 1874, p. 5
Megabacteria Billroth, 1874, p. 16
Mesobacteria Billroth, 1874, p. 16
Microbacteria Billroth, 1874, p. 16
Monobacteria Billroth, 1874, p. 16
Petalobacteria Billroth, 1874, p. 16
Polybacteria Van Tieghem, 1884, p. 1114
Punctula Van Tieghem, 1884, p. 1114
Diplobacterium Hasse, 1887, p. 347
Gliscrobacterium Malerba and Sanna Salaris, 1888, p. 486
Photobacter Beijerinck

The following names have been designated as genera, but without species.

Arthrobacterium De Bary, 1884
Arthrobacter Fischer, 1895, p. 141
Arthrobactridium Fischer, 1895, p. 140
Arthrobactrillum Fischer, 1895, p. 139
Arthrobactrinium Fischer, 1895, p. 139
Thermobacterium Fuhrmann, 1906, p. 8

The following generic names are old algal or protozoan genera to which bacteria were occasionally ascribed.

Monas Mueller, 1773, p. 3
Gloeotila Kuetzing, 1843, p. 245
Mycothamnion Kuetzing, 1843, p. 126

The following are aberrant spellings of generic names.

Kokkobacillus Biedert, 1885, p. 439
Arthrobakterium Hueppe, 1886, p. 145
Glischrobacterium Rothmann, 1904, p. 491
Bakterium of many German authors

The following are subgenera:

Eu-Klebsiella Trevisan, 1889, p. 1028
Eu-Mantegazzaea Trevisan, 1889, p. 942
Eu-Pacinia Trevisan, 1889, p. 23
Eupseudomonas Migula, 1895, p. 29

The following names are not invalid for any of the preceding reasons.

Mycoderma Persoon, 1822, p. 96
Serratia Bizio, 1823
Zaogalactina Sette, 1824, p. 51
Bacterium Ehrenberg, 1828, p. 8
Ulvina Kuetzing, 1837, p. 26
Umbina Naegeli, 1849
Zoogloea Cohn, 1854, p. 123
Schinzia Frank, 1879, p. 376
not *Schinzia* Dennstatt, 1818
not *Schinzia* Naegeli, 1842, p. 279
Mantegazzaea Trevisan, 1879, p. 146
Cromobacterium Bergonzini, 1881, p. 153
Tyrothrix Duclaux, 1879, p. 79
Actinobacter Duclaux, 1882, p. 110
Dispora Kern, 1882, p. 135
Bacteriopsis Trevisan, 1885, p. 103
Coccobacillus Leube, 1885
Klebsiella Trevisan, 1885, p. 94
Kurthia Trevisan, 1885, p. 92
Octopsis Trevisan, 1885, p. 102
Proteus Hauser, 1885, p. 100
Helicobacterium Miller, 1886, p. 117
Phytomyza Schroeter, 1886, p. 134
Diplobacillus Weichselbaum, 1887, p. 212
Pasteurella Trevisan, 1887, p. 94
Zygobacterium Maggio, 1887, p. 318
Cladochytrium Vuillemin 1888, p. 121
Coccobacterium Rivolta, 1888.
Diccoccia Trevisan 1889, p. 26

Photobacterium Beijerinck 1889, p. 401
Pneumobacillus Arloing, 1889, p. 428
Rhizobium Frank, 1889, p. 338
Winogradskya Trevisan, 1889, p. 12
Ascobacterium Babes, 1890, p. 155
Nevskia Famintzin, 1891, p. 481
Pseudomonas Migula, 1894, p. 237
Halibacterium Fischer, 1894, p. 22
Rhizobacterium Kirchner 1895, p. 213
Oenobacillus Forti, 1896, fasc. 1
Corynebacterium Lehmann and Neumann, 1896, p. 350
Astrobacter Jennings, 1896, p. 312
Aërobacter Beijerinck, 1900, p. 198
Pseudorhizobium Hartleb, 1900, p. 887
Azotobacter Beijerinck, 1901, p. 561
Corynethrix Bongert 1901, p. 449
Lactobacillus Beijerinck 1901b, p. 214
Lactobacter Beijerinck 1901b, p. 200
Brachybacterium Troili-Peterson, 1903, p. 138
Thiobacillus Beijerinck, 1904, p. 598
Acetobacter Fuhrmann, 1905, p. 8
Erythrobacillus Fortineau, 1905, p. 104
Pyobacillus Koppanyi, 1907, p. 429
Acetimonas Jensen, 1909a, p. 312
Carboxydomonas Jensen, 1909a, p. 311
Caseobacterium Jensen, 1909a, p. 311
Corynemonas Jensen, 1909a, p. 344
Denitrobacterium Jensen, 1909a, p. 314
Denitromonas Jensen, 1909a, p. 314
Erysipelothrix Rosenbach, 1909, p. 367
Liquidobacterium Jensen, 1909a, p. 337
Liquidomonas Jensen, 1909a, p. 332
Methanomonas Jensen, 1909a, p. 311
Propionibacterium Jensen, 1909a, p. 337
Rhizomonas Jensen, 1909a, p. 334
Thermobacillus Jensen, 1909a, p. 339
Asterococcus Borrel, etc., 1910, p. 179

Fusiformis Hoelling, 1910, p. 240
Thermobacterium Lindner
Microbacillus Sabouraud
Photobacter Beijerinck
Ascobacillus Unna and Tommasoli, p. 60

Among this list of generic names the following are based upon species insufficiently described, and are disregarded here: *Actinobacter, Astrobacter, Coccobacterium, Helicobacterium, Winogradskya, Zoogloea, Zygobacterium.*

The following have been termed physiological genera and have usually been regarded as invalid: *Halibacterium, Photobacter, Photobacterium, Lactobacter.*

The following generic names have not been included for other reasons: *Ascobacillus, Ascobacterium, Carboxydomonas, Oenobacillus, Photobacterium, Methanomonas, Microbacillus.*

The following key gives the principal differential characters of the subtribes which are proposed:

Key to the subtribes of the Bacterieae

A. Cells usually fusiform.....Subtribe I. *Fusiforminae*
B. Cells not fusiform.
  I. Requiring serum or hemoglobin for development. Obligate parasites. Gram-negative. Non-motile..Subtribe II. *Hemophilinae*
  II. Not requiring serum, or at least hemoglobin for development. Gram-negative or positive. Motile or non-motile.
    a. Obligate aërobes, securing growth energy by oxidation of carbonaceous compounds, as carbohydrates, alcohols, etc.....Subtribe III. *Rhizobiinae*
    b. Not obligate aërobes, not securing growth energy by oxidation of carbonaceous compounds, Subtribe IV. *Bacteriinae.*

Subtribe I. **Fusiforminae** Subtrib. nov.

This tribe has a single genus, *Fusiformis* Hoelling.

Genus I. **Fusiformis** Hoelling 1910, p. 240

Synonym:
Mantegazzaea Vuillemin 1913, p. 521
not *Mantegazzaea* Trevisan 1879, p. 137

Obligate parasites. Cells usually elongate and fusiform. Gram-negative? Anaërobic. Non-motile. No spores. In some respects approaching the spirochetes in morphology.

The type species is *Fusiformis termitidis* Hoelling (possibly *F. dentium*).

Vuillemin (1913, p. 521) has proposed that the name *Mantegazzaea* Trevisan be retained as one of the "formogenera conservanda," with the fusiform bacillus of the medical writers as the type species. The name as used by Trevisan was used principally for certain of the sulphur bacteria, and not for forms related to this type.

Subtribe II. **Hemophilinae** Subtrib. nov.

Strict parasites, requiring hemoglobin or at least serum for their growth in media. Gram-negative. Non-motile. Cells may be pleomorphic. Usually very small. No spores.

The two genera may be differentiated by the following key:

Key to the genera of Hemophilinae

A. Requiring serum for growth. Cells almost ultra-microscopic. Stain best by Giemsa. Involution forms characteristic.....Genus 1. *Asterococcus*

B. Requiring hemoglobin for growth. Stain readily with ordinary aniline dyes. Involution forms infrequent.....Genus 2. *Hemophilus*

Genus I. **Asterococcus** Borrel, *et al.*, 1910, p. 179

Pleomorphic cells, appearing at different stages of development as isolated cocci or as chains of cocci, as rods and as filaments variously branched and swollen. Very minute, almost ultra-microscopic. Non-motile. No spores. Stains with difficulty, best with Giemsa stain. Growth in cultures only in presence of serum or of hemoglobin.

The type species is *Asterococcus mycoides* Borrel, *et al.*, of bovine pleuropneumonia.

Genus 2. **Hemophilus.** Committee

Rod-shaped cells, minute, non-motile, without spores, strict parasites, growing best or only in presence of hemoglobin. Gram-negative, stain readily with aniline dyes.

The type species is *Hemophilus influenzae*, (——) comb. nov. the cause of influenza.

Subtribe III. **Rhizobiinae** Subtrib. nov.

Rod-shaped organisms, securing their growth energy by the oxidation of carbonaceous compounds, as carbohydrates, alcohol, etc. Do not require serum, etc. Not parasitic in animals.

The following key gives the principal differential characters of the genera:

Key to the genera of Rhizobiinae

A. Not fixing atmospheric nitrogen; securing growth energy usually by the oxidation of ethyl alcohol to acetic acid.....Genus 1. *Mycoderma*

B. Capable of fixing appreciable amounts of atmospheric nitrogen. Grow well on nitrogen-free media.

a. Small motile rods, with abundant involution forms, frequently living in root nodules of the higher plants (legumes)...Genus 2. *Rhizobium*

b. Not symbiotic. Cells larger, plump, almost spherical in some cases. Genus 3. *Azotobacter*

Genus I. **Mycoderma** Persoon, 1822, p. 96 emended

Synonyms:

Ulvina Kuetzing, 1837, p. 26
Umbina Naegeli, 1849
Bacteriopsis? Trevisan, 1885, p. 103
Acetobacter Fuhrmann, 1905, p. 8
Acetimonas Jensen, 1909a, p. 312

Cells rod-shaped, frequently in chains, non-motile usually, without spores. Obligate aërobes, growing usually as a film on the surface of alcoholic solutions, transforming the alcohol to acetic acid. Involution forms often developed and quite characteristic.

The type species is *Mycoderma aceti* Thompson?

There is some doubt as to the appropriateness of *Mycoderma* as the name of this genus. It is possible that it should be reserved for the yeasts.

Genus 2. **Rhizobium** Frank, 1889, p. 338

Synonyms:

Cladochytrium Vuillemin, 1888, p. 121
Phytomyxa Schroeter, 1886, p. 134
Schinzia Denstatt, 1818, in part
Rhizobacterium Kirchner, 1895, p. 213
Pseudorhizobium Hartleb, 1900, p. 887
Rhizomonas Jensen, 1909a, p. 334

Rod-shaped cells, motile when young by means of polar flagella, obligate aërobes, fixing atmospheric nitrogen when grown in a nitrogen-free medium containing suitable carbohydrates, involution forms abundant and characteristic, usually growing in the nodules of the roots of leguminous plants.

The type species is *Rhizobium leguminosarum* Frank.

The name of this genus has been a source of confusion. The organism of leguminous nodules was placed in the mold genus *Schinzia* by Frank as *Schinzia leguminosarum*. Schroeter (1886, p. 134) concluded the organism to be one of the slime molds and created the genus *Phytomyxa*, including it in the order *Phytomyxini* among the *Myxomycetes*. He based his conclusions as to the position of this organism among the slime molds upon the work of Prilleaux (1879, p. 98). Beijerinck (1888, p. 758) named the organism *Bacillus radicicola*. Frank (1889, p. 338) renamed the organism *Rhizobium leguminosarum*. The fact that Schroeter included this genus incorrectly among the slime molds does not invalidate the name. The Committee on Classification of Bacteria, of the Society of American Bacteriologists, however, has recommended the use of the generic name *Rhizobium* as better known and probably resulting in less confusion than the use of *Phytomyxa*.

Genus 3. **Azotobacter** Beijerinck, 1901d, p. 561

Relatively large rods, or even cocci, sometimes almost yeast-like in appearance, dependent primarily for growth energy upon the oxidation of carbohydrates; obligate aërobes, usually growing

in a film upon the surface of the culture medium. Capable of fixing atmospheric nitrogen in considerable amounts when grown in solutions deficient in combined nitrogen. Motile or non-motile, if the latter, with polar flagella.

The type species is *Azotobacter chroöcoccum* Beijerinck.

It is possible that the demonstration of endospore production by Löhnis and his co-workers will require the removal of this genus to the *Bacilleae*.

Subtribe IV. **Bacteriinae** Subtrib. nov.

Cells not fusiform, rod shaped; not hemoglobinophilic; aërobic, facultative, or microaërophilic; not securing growth energy exclusively by the oxidation of carbonaceous compounds. Spores never formed. Motile or non-motile.

The following key to the genera recognized gives the most important differential characters:

Key to genera of Bacteriinae

A. Producing usually a yellowish or greenish or fluorescent pigment, usually Gram-negative, motile by means of polar flagella, or non-motile.
Genus 1. *Pseudomonas*

B. When pigmented not greenish or fluorescent, when motile with peritrichous flagella.
1. Cells typically pigmented, chromoparous.
a. Producing red or pink pigment.....Genus 2. *Serratia*
b. Producing a violet pigment.....Genus 3. *Chromobacterium*
2. Cells not typically definitely pigmented, or at least not red or violet.
a. Cells typically Gram-negative.
(1) Non-motile, showing bipolar staining commonly. Never produce gas from carbohydrates. Power of acid production low.....Genus 4. *Pasteurella*
(2) Not showing bipolar staining.
(a) Not producing honey-like growth on potato, branching forms uncommon.
x. Gelatin not liquefied or liquefied very slowly. Motile or non-motile.....Genus 5. *Bacterium*
xx. Gelatin liquefied quickly. Motile.
Genus 6. *Proteus*
(b) Producing a honey-like growth on potato. Branched cells not uncommon.
Genus 7. *Pfeifferella*

b. Cells typically Gram-positive. All non-motile.
(1) Usually microaërophilic. Not typically growing well on the surface of laboratory media. Without metachromatic granules.
(a) Non-pathogenic lactic acid bacilli. Genus 8. *Lactobacillus*
(b) Pathogenic, slender, small rods, not lactic acid formers..... Genus 9. *Erysipelothrix*
(2) Aërobic rods, frequently showing metachromatic granules or irregular staining...Genus 10. *Corynebacterium*

Genus 1. **Pseudomonas** Migula, 1894, p. 237, emended

Synonyms:

Bacterium Ehrenberg emended Cohn, 1872, p. 167
Bactrillum Fischer, 1895, p. 139
Arthrobactrinium Fischer, 1895, p. 139
Arthrobactrillum Fischer, 1895, p. 139
Bactrinius Kendall, 1902, p. 484
Bactrillius Kendall, 1902, p. 484
Denitromonas Jensen, 1909, p. 314
Liquidomonas Jensen, 1909, p. 332

Rod-shaped bacteria, never spiral, usually motile by means of polar flagella or rarely non-motile. Aërobic and facultative. Frequently liquefying gelatin. Without spores. Gram stain variable. Usually producing a water-soluble pigment which diffuses through the medium as a green, blue, purple, or brown or in some cases a yellow pigment. Fermentation of carbohydrates usually not active.

The type species is *Pseudomonas aeruginosa* (Schroeter) Frost?

It is difficult to determine whether the generic names *Pseudomonas* or *Bacterium* should be used here. There is evidence that the latter is historically valid for this genus, but it has not generally been so accepted. The generic name *Bacterium* has been used in some seven different senses.

1. Bacterium. The conception held before the work of Cohn, 1872. Relatively rigid cells or chains of cells, not flexible, motile, motion usually oscillatory. The name was used in this sense by Ehrenberg (1828), Dujardin (1841), Perty (1852) and Davaine (1868).

2. Bacterium. Short cylindric or elliptic cells, never in chains or filaments, showing stages of motility and non-motility. Cohn (1872b, 1875), Dallinger and Drysdale (1875), Magnin (1878), Winter (1879), Luerssen (1879), Grove (1884), Van Tieghem (1884), Flügge (1886).

3. Bacterium. Rod-shaped organisms which do not produce endospores. Motility and cell groupings not emphasized. The genus is contrasted with *Bacillus* in these characters. Zopf (1885), Hueppe (1885), Schroeter (1886), De Toni and Trevisan (1889), Ludwig (1892), Freudenrich (1894), Lehmann and Neumann (1896), Löhnis (1913).

4. Bacterium. Not used as a generic name, all rod-shaped organisms being placed in *Bacillus* or other genera. Baumgarten (1890), Sternberg (1892), Fischer (1897), Matzuschita (1902), Schneider (1912).

5. Bacterium. Non-motile rod-shaped organisms. Migula (1895, 1897, 1904), Chester (1901), Smith (1902), Kendall (1902), Ellis (1909), Heim (1911).

6. Bacterium. Polar flagellate organisms of the type of the fluorescent bacilli. E. F. Smith (1906), Vuillemin (1913).

7. Bacterium. Organisms belonging to the colon-typhoid group. Orla Jensen (1909a).

This diversity in definition of this genus has led to considerable confusion in literature. It would seem logical to select one of the species early named *Bacterium*, and designate it as the generic type. However, of the species named before Cohn's descriptions in 1872 there does not seem to be a single one which can be identified with certainty.

It might seem best to select some organism early named *Bacterium* as the type and so word the generic diagnosis as to include this form and the related species.

E. F. Smith has attempted to identify the *Bacterium termo* of Cohn, evidently intended by this author to constitute the type of the genus. He states

> His (Cohn's) *Bacterium termo* was a small schizomycetous organism capable of growing freely in Cohn's nutrient solution, containing acid potassium phosphate and ammonium tartrate. It produced thereon

short rods (single, in pairs or fours, joined end to end) and roundish lobed white zoogloeae, together with a *greenish* fluorescence. It did not appear in boiled fluids, i.e. was destitute of endospores (Cohn) and the motile rods were killed by short exposure to 58°C. (Schröeter). In other words, it was a non-sporiferous green fluorescent organism possessed of a single polar flagellum, or in some cases, perhaps, provided with paired or triple polar flagella.

Smith attempted to follow Cohn's procedure in getting cultures of *Bact. termo* and succeeded, by inoculating Cohn's solution with water in which beans had been thrown, in securing a green fluorescent organism with a polar flagellum. He concludes that the morphologically similar non-fluorescent forms and the yellow bacteria should be included in this genus.

Vuillemin by an entirely distinct line of reasoning came to a similar conclusion. He notes that Ehrenberg based his generic description in part upon the type of cell motility, oscillatory. Today, he states, we know this characteristic type of motion to be due to the presence of polar flagella on short rods. He concludes that the *Bact. termo* is closely related to *Bacillus pyocyaneus* and accepts this as the type of the genus.

Still a third line of evidence will lead to a similar conclusion, i.e., a study of the species of *Bacterium* other than *Bact. termo* recognized by Cohn and his coworkers. Three species previously described were placed in the genus *Bacterium* by Schröeter. One of these was the *Vibrio syncyaneus* of Ehrenberg, the organism repeatedly described in literature as the cause of blue coloration in milk. It is a polar flagellate rod. The name *Bact. aeruginosum* is the earlier name applied by Schröter to the blue pus organism, chosen by Vuillemin to constitute the generic type. The organism termed *Bact. xanthinum*, however, belongs to an entirely distinct group.

It is evident therefore that there is historical justification for E. F. Smith's emendation of the genus. The principal criticism is the undue insistence upon motility. It would seem that an organism morphologically similar but non-motile, showing the same fluorescence and physiological characters should likewise be included in the genus.

Notwithstanding the force of the above arguments in favor of using the generic name *Bacterium* for this group, this use will lead to the greatest confusion in the literature. It would seem best to use the name *Pseudomonas* for this genus, designating *Bacterium* as a *genus conservandum* for the colon-typhoid group of bacteria in accordance with the proposal of Orla Jensen (1909a p. 343). This is the recommendation of the Committee on Classification of the Society of American Bacteriologists.

Genus 2. **Serratia** Bizio, 1823, emended

Synonyms:

Zaogalactina Sette, 1824, p. 51

Erythrobacillus. Fortineau, 1905, p. 104

Cells rod-shaped, without spores. Motile by means of peritrichous flagella or non-motile. Gram stain variable. Aerobic, producing a red or pink pigment, a lipochrome. Possibly closely related yellow and orange lipochrome-forming bacteria should be included here as well.

The type species is *Serratia marcescens* Bizio, the organism usually termed *Bacillus prodigiosus.* The synonomy of this organism is as follows:

Serratia marcescens Bizio, 1823

Zaogalactina imetropha Sette, 1824, p. 51

Monas prodigiosa Ehrenberg, 1848

Palmella prodigiosa Montagne, p. 727

Micrococcus prodigiosus Cohn, 1872a, p. 153

Bacillus prodigiosus Fluegge, 1886, p. 284

Bacillus imetrophus Trevisan, 1887

Bacillus marcescens De Toni and Trevisan, 1889, p. 976

Pfeiffer (1887, p. 46) lists *Serratia* under *Fungi dubiae sedis* and ascribes the genus to *Bergamaschi.*

Vuillemin (1913, p. 521) concluded that although the characters used by Bizio have no generic value, nevertheless the generic name might well be revived to include the rod-shaped bacteria with diffuse flagella. He terms *Serratia subtilis* (*Bacillus subtilis*) the type of his emended *genus conservandum.*

Genus 3. **Chromobacterium** Bergonzini, 1881, p. 153

Synonyms. The name was spelled *Cromobacterium* by Bergonzini, and corrected by Zimmerman (1881 p. 1528). The spelling *Chromobacterium* has been accepted by other writers. (Grove, 1884, p. 26; De Toni and Trevisan, 1889, p. 978).

Rod-shaped bacteria, without spores, aerobic, producing a violet chromoparous pigment soluble in alcohol but not in chloroform, motile or non-motile, Gram stain variable.

The type species is *Chromobacterium violaceum* Bergonzini.

Genus 4. **Pasteurella** Trevisan, 1887, p. 94

Synonyms:

Octopis? Trevisan, 1885, p. 102
Coccobacillus Gamaleia, 1888, p. 167
not *Coccobacillus* Leube, 1885
Dicoccia? Trevisan, 1889, p. 1034
Diplobacillus? Weichselbaum, 1887, p. 212

Short rods, single or rarely in chains, usually showing distinct polar staining, non-motile. Gram-negative, without spores, aerobic and facultative, usually not producing gas, powers of fermentation slight, often pathogenic, not acid-fast, not liquefying gelatin.

The type species is *Pasteurella choleraegallinarum* (Fluegge) Trevisan.

Genus 5. **Bacterium** Ehrenberg, 1828, emended, Jensen, 1909

Synonyms:

Actinobacter Duclaux, 1882, p. 110
Klebsiella Trevisan, 1885, p. 94
Kurthia? Trevisan, 1885, p. 92 in part
Pneumobacillus? Arloing, 1889, p. 428
Pyobacillus Koppanyi, 1907, p. 429
Aërobacter Beijerinck, 1900, p. 198
Salmonella Lignieres, 1900a, p. 389
Tyrothrix? Duclaux, 1879, in part?

Plump rods, without spores, Gram-negative, motile by means of peritrichous flagella, or non-motile, liquefying gelatin very slowly or not at all. Usually showing marked power to ferment carbohydrates, frequently with gas production.

The type species is *Bacterium coli* Escherich.

The genus is a large one, including many species. It is generally divided into subgroups based primarily upon the fermentative reactions. It may be convenient to recognize these as subgenera separated from each other by the characteristics noted in the following key:

Key to the subgenera of Bacterium

A. Organisms which show a maximum of fermentative power, including fermentation of lactose, rarely pathogenic, some forms slowly liquefy gelatin. Subgenus 1. *Aërobacter (or Eu-Bacterium).*

B. Organisms not showing maximum fermentative power, never producing gas in lactose, frequently pathogenic, never liquefying gelatin.

1. Producing acid and gas from glucose, sometimes other sugars, but not from lactose..... Subgenus 2. *Salmonella*
2. Producing gas from none of the carbohydrates, acid sometimes formed..... Subgenus 3. *Eberthella*

Subgenus 1. **Aërobacter** Beijerinck, 1900, p. 198

Fermenting both glucose and lactose with formation of both acid and gas. Pathogenicity slight.

Type species is *Bacterium (Aerobacter) coli* Escherich.

Subgenus 2. **Salmonella,** Lignieres,

Fermenting glucose but not lactose with formation of acid and gas. Frequently pathogenic.

The type species is *Bacterium (Salmonella) cholerae suis*?

Subgenus 3. **Eberthella** subgen. nov.

Not producing gas from any of the carbohydrates, acid may or may not be formed.

The type species is *Bacterium (Eberthella) typhi.* Flügge.

Genus 6. **Proteus** Hauser, 1885, p. 1

Synonyms:

Liquidobacterium Jensen, 1909a, p. 337
Spirulina Hueppe, 1886, p. 146
not *Spirulina*, Turpin, 1827

Short rods, showing great variation in morphology, filamentous and bent rods as involution forms frequent. Motile by means of peritrichous flagella. The species commonly produce motile "islands" on the surface of moist solid media. No spores. Gram-negative. Usually liquefying gelatin rapidly in absence of carbohydrates. Usually producing acid and gas from certain carbohydrates. In general the species are closely associated with decay and putrefaction, sometimes pathogenic.

The type species is *Proteus vulgaris* Hauser.

Genus 7. **Pfeifferella** gen. nov.

Non-motile rods, slender, Gram-negative, without spores, staining poorly, sometimes forming threads and showing a tendency toward branching. Gelatin may be slowly liquefied. Do not ferment carbohydrates. Growth on potato characteristically honey-like.

The type species is the glanders bacillus, *Pfeifferella mallei.*

Genus 8. **Lactobacillus** Beijerinck, 1901, p. 214

Synonyms:

Lactobacter Beijerinck
Brachybacterium Troili Petersson, 1903, p. 138 in part
Caseobacterium Jensen, 1909a, p. 478
Dispora? Kern, 1882a, p. 135
Tyrothrix? Duclaux, 1882, in part

Rod-shaped organisms, cells frequently quite elongate, non-motile, without spores, Gram-positive in young cultures. Produce acid, largely lactic, from carbohydrates. When gas is produced, it is CO_2 without hydrogen. For the most part the organisms are thermophilic. Microaërophilic.

The type species is *Lactobacillus caucasicus* (*Kern*) *Beijerinck.*

Genus 9. **Erysipelothrix** Rosenbach, 1909, p. 367

Rod-shaped organisms with a tendency to the formation of long filaments which may show branching. The filaments may also thicken and show characteristic granules. No spores. Non-motile. Gram-positive. Does not produce acid. Microaërophilic. Usually parasitic.

The type species is *Erysipelothrix rhusiopathiae* (*E. porci*), the causal organism of swine erysipelas.

Genus 10. **Corynebacterium** Lehmann and Neumann, 1896

Synonyms:

Corynemonas Jensen, 1909, p. 22
Corynethrix Bongert, 1901, p. 449

Rods which stain interruptedly (striped) with weak staining solutions. Not acid-fast. Clubbed, wedge-shaped and pointed rods frequent. Gram-positive. Non-motile. No spores. Aërobic.

The type of the genus is *Corynebacterium diphtheriae* Lehmann and Neumann.

Tribe III. **Mycobacterieae** Trib. *nov.*

Synonyms:

Coccothrichaceen Lutz, 1886, p. 22
Mycobacteriaceae Chester, 1897, p. 62 in part

Rod-shaped organisms occasionally showing a tendency to branching, acid-fast, Gram-positive, non-motile, without spores.

A single genus is included, *Mycobacterium* Lehmann and Neumann.

Genus 1. **Mycobacterium** Lehmann and Neumann, 1896, p. 363

Synonyms:

Coccothrix Lutz 1886, p. 22
Sclerothrix Metschnikoff, 1888, p. 70
not *Sclerothrix* Kuetzing, 1849, p. 319

Slender rods which stain with difficulty, but when once stained, are acid-fast. Clubbed, swollen, clavate or cuneate cells occur, even filaments with branches. Non-motile. Without spores. Gram-positive.

The type species is *Mycobacterium tuberculosis* (Koch) Lehmann and Neumann.

Vuillemin (1913 p. 527) contends that *Coccothrix* resembles too closely *Coccotrichum* Wallroth. It does not seem that the resemblance is close enough ever to cause confusion, and the genus name *Coccothrix* is apparently valid. The Committee on Classification of the Society of American Bacteriologists, however, has recommended the use of the generic name *Mycobacterium* as better known and less likely to result in confusion.

REFERENCES

Arloing, S. 1889 Sur l'étude bacteriologique des lesions de la peripneumonie contagieuse du boeuf. Compt. rend. hebd. des Seances de l'Academie des Sciences, **109**, 428.

Babes, V. 1890 *In*, Les Bacteriees, Cornil, A. V. and Babes, V., **1**, 155.

Baumgarten, Paul V. 1890 Lehrbuch der pathologischen Mykologie. Leipzig, 127.

Beijerinck, M. W. 1889 Le *Photobacterium luminosum*. Bacterie lumineuse de la mer du nord. Arch. Neerlandaises des Sciences exact. et nat., **23**, 401.

Beijerinck, M. W. 1893 Ueber die Butylalkoholgärung und das Butylferment. Verhandl. der Konmklijke Akademie van Wetenschappen te Amsterdam, Sect. II, Deel I, No. 10.

Beijerinck, M. W. 1894 Ueber die Butylalkoholgärung und das Butylferment. Abs. Cent. f. Bakt., **15**, 171.

Beijerinck, M. W. 1900 Schwefelwasserstoffbildung in den Stadtgräben und Aufstellung der Gattung Aërobacter. Cent. f. Bakt., II, Abt. **6**, 193–206.

Beijerinck, M. W. 1901a Sur la formation de l'hydrogen sulfure dans les canaux et le genre nouveau Aërobacter. Arch. Neerl. Sci. Holl., Ser. 2, **4**, 1–18. Haarlem.

Beijerinck, M. W. 1901b Sur les Ferments Lactiques de l'Industrie. Arch. Neerlandaises des Sciences Exact. et Nat., Ser. 2, **6**, 213.

Beijerinck, M. W. 1901c Anhaufungsversuche mit Ureumbakterien, Ureumspaltung durch Urease und durch Katabolismus. Cent. f. Bakt., II Abt., **7**, 33.

Beijerinck, M. W. 1901d Ueber oligonitrophile Mikroben. Cent. f. Bakt., II Abt., **7**, 561–582.

BEIJERINCK, W. M. 1904 Ueber die Bakterien welche sich im Dunkeln mit Kohlensäure als Kohlenstoffe ernähren können. Cent. f. Bakt., II Abt., **11,** 598.

BERGONZINI, CURZIO 1881 Sopra un nuovo Batterio Colorato. Annuario della Societa dei naturalisti in Modena. Ser. 2, **14,** 149–158.

BIEDERT, PH. 1885 Beitrag zur Frage nach der Constanz der Spaltpilze. (*Kokkobacillus zymogenus* and *Bacterium termo.*) Virchow's Arch., **100,** 439.

BILLET, A. 1890 Contribution a l'étude de la Morphologie et du Developpement des Bacteriacees. Extrait du Bulletin scientifique de la France et de la Belgique. Paris.

BILLROTH, THEODOR 1874 *Coccobacteria septica.*

BIONDI, D. 1887 Die pathogenen Mikroorganismen des Speichels. Ztschr. f. Hyg., **2,** 192–236.

BIZIO, B. 1823 Polent. porporp. Bibl. ital., **30,** 288.

BONGERT 1901 Corynethrix pseudotuberculosis murium ein neuer pathogener Bacillus für Mäuse. Ztschr. f. Hyg., **37,** 449–475.

BORREL, DUJARDIN-BEAUMETZ, JEANTET AND JOUAN. 1910 Le Microbe de la Peripneumonie. Annales de l'Inst. Pasteur, **24,** 179.

CHATTON AND PERARD 1913 Schizophytes du coecum du cobaye. II Metabacterium polyspora n. g. n. sp. compt. rend. hebd. des seances et mem. de la Soc. de Biologie, **65,** 1232.

CHESTER, F. D. 1897 Classification of the Schizomycetes. Ninth Annual Report Delaware College Agr. Exp. Sta., pp. 62–63.

CHESTER, F. D. 1901 Manual of determinative bacteriology.

COHN, FERDINAND 1854 Untersuchungen über die Entwickelungsgeschichte der mikroskopischen Algen und Pilze. Der Akademie übergeben den 1 Mai, 1853. Nov. Act. Acad. Caes. Leop.-ü Carol. Nat. Cur., **24,** 103–256.

COHN, FERDINAND 1872a Organismen in der Pockenlymphe. Virchow's Arch., **55,** 229–238 (a).

COHN, FERDINAND 1872b Untersuchungen über Bakterien. Beiträge zur Biologie der Pflanzen, **1** (Heft 1), 127–224 (b).

COHN, FERDINAND 1875 Untersuchungen über Bakterien. II Beiträge z. Biologie d. Pflanzen, **1** (Heft 3), 141–208.

CONN, H. W. 1909 Agricultural Bacteriology, 2 ed., p. 12.

CORNIL AND BABES 1890 Les Bacteries.

DALLINGER, W. H. AND DRYSDALE, J. J. 1875 On the existence of flagella in *Bact. termo.* Monthly Microscopical Journal, p. 105.

DANGEARD, A. 1891 Contribution a l'étude des Bacteriacees vertes (Eubacillus nov. gen.). Le Botaniste, **2,** 151.

DAVAINE, C. 1868 Dictionnaire Encyclopedique des Sciences Medicales, Ser I, **8,** 21.

DE BARY, A. 1884 Vergl. Morph. u. Biol. d. Pilze. Mycetozoa und Bacterien. Leipzig.

DENNSTÄTT 1818, (according to Schroeter, l. c., p. 134)

DETONI, J. B. AND TREVISAN, V. 1889 Schizomycetaceae Naeg. in Saccardo's Sylloge Fungorum. **8,** 923–1087.

DUCLAUX, E. 1882–1883 Deuxieme memoire sur le lait. Ann. de l'Inst. Agron., **7,** 35.

DUJARDIN, F. 1841 Histoire naturelle des Zoophytes. Paris.
EHRENBERG, C. G. 1828 Symbolae Physicae, Animalia Evertebrata.
EHRENBERG, C. G. 1848 Berichte über die Verhandlungen der Königliche Preuss. Akademie zu Berlin, 349, 354, 384, 462.
EHRENBERG, C. G. 1849 Ibid., 101.
EHRENBERG, C. G. 1850 Ibid., 364.
ELLIS, DAVID 1909 Outlines of Bacteriology, p. 6.
FICK 1887 Ueber Mikroorganismen im Conjunctivalsack. Wiesbaden.
FISCHER, ALFRED 1895 Untersuchungen über Bakterien. Jahresb. f. wiss ensch. Bot., **27**, 1–163.
FISCHER, ALFRED 1903 Vorlesungen über Bakterien. 2d ed.
FLÜGGE, C. 1908 Grundriss der Hygiene, Ed. 6, p. 514.
FORTI, C. 1896 Notizie complementari su alcuni studi di zimotecnia enologica esegwiti a tutto il. Ann. della soc. chim. di Milano, vol. **3**, fasci. 1.
FORTINEAU, L. 1905 *L'Erythrobacillus pyosepticus*. Compt. rend. Soc. biol., **58**, 104.
FRANK, B. 1879 Ueber die Parasiten in den Wurzelausschwellungen der Papilionaceen. Bot. Zeitung **37**, 376.
FRANK, B. 1889 Ueber die Pilzsymbiose der Leguminosen. Ber. d. deut. bot. Gesellsch. **7**, 338.
FREUDENREICH 1894 Les Microbes et leur role dans la laiterie.
FUHRMANN, F. 1905 Morphologisch.-biologische Untersuchungen über ein neues Essigsäuresbildendes Bakterium. Beiheft zum. bot. Centralbl. **19**, 1–31.
GAMALEÏA, N. 1888 Zur Aetiologie der Hühnercholera. Centralbl. f. Bakt. **4**, 167.
GONNERMANN, M. 1907 Ueber gallertbildende Bakterien. Oesterungar. Ztschr. f. Zuckerind. u. Landwirtsch. **36**, 877–888.
GROVE, W. B. 1884 A Synopsis of the Bacteria and Yeast Fungi.
HARTLIEB, R. 1900 Die Morphologie und systematische Stellung der sogenannten Knöllchenbakterien. Chem. Zeitung. **24**, 887.
HAUSER 1885 Fäulnisbakterien.
HEIM, LUDWIG 1911 Lehrbuch der Bakteriologie, 4 ed. p. 250.
HEWLETT, R. I. 1898 A Manual of Bacteriology, p. 18. Philadelphia.
HOELLING 1910 Die Kernverhältnisse von *Fusiformis termitidis*. Arch. f. Protistenkunde **19**, 240.
HUEPPE, F. 1886 Die Formen der Bakterien und ihre Beziehungen zu den Gattungen und Arten. Wiesbaden.
ITZIGSOHN 1867 Sitz. Ges. nat. Fr. zu Berlin. Novem. According to Koch in Cohn's Beiträge II, 3, p. 414.
JENSEN, ORLA 1909a Die Hauptlinien des natürlichen Bakteriensystems. Centralbl. f. Bakt., II Abt., **22**, 305–346.
JENSEN, ORLA 1909b Vorschlag zu einer neuen bakteriologischen Nomenklatur. Centralbl. f. Bakt., II Abt., **24**, 477.
KENDALL, ARTHUR I. 1902 A Proposed Classification and Method of Graphical Tabulation of the Characters of Bacteria. Public Health. **28**, 484.
KERN, EDWARD 1882a Dispora caucasica. Nov. gen. et. nov. sp. eine neue Bakterienform. Biol. Zentralblatt **2**, 135.

KERN, EDWARD 1882b Ueber ein neues Milchferment aus dem Kaukasus. Bull. de la Soc. Imperiale des Naturalistes de Moscou. 2d Part, **56**, 141–173.

KIRCHNER, O. 1895 Die Wurzelknöllchen der Sojabohne. Cohn's Beitr. zur Biol. d. Pflanzen **7**, 213.

KLEBS, E. 1871 Die Ursachen der Infectiösen Wundkrankheiten. Correspondenzbl. f. Schweizer Aerzte. ?

KOPPANYI, EMERICH 1907 Ueber eine mit fibrinösen Pleuritis einhergehende Pyämie der Kaninchen. Ztschr. f. Tiermed. Jena. **11**, 429–454.

KÜTZING, FRIEDRICH 1904 Mikroskopische Untersuchungen über die Hefe und Essigmutter, nebst mehreren anderen dazu gehörigen vegetabilischen Gebilden. Auszuge vorgetragen in der Versammlung des naturhistorischen Vereins des Harzes in Alexisbad am 26 Juli 1837. Reprinted Delbrück, M. and Schrohe, A. Hefe, Gärung und Fäulnis. pp. 25–38.

KÜTZING, F. T. 1849 Species Algarum.

LEHMANN, K. B. AND NEUMANN, R. 1896 Atlas und Grundriss der Bakteriologie München, vol. 1 and 2.

LEUBE 1889 *In* Virchows Arch. B. C. fasc. 3. 1885. According to Saccardo, **8**, 966.

LÖHNIS, F. 1913 Vorlesungen über landwirtschaftliche Bakteriologie, p. 45.

LOTSY, J. P. 1907 Vorträge über botanische Stammesgeschichte. Vol. I, Algen und Pilze.

LUDWIG, FRIEDRICH 1892 Lehrbuch der niederen Kryptogamen.

LUERSSEN, CHR. 1879 Botanik. **1**, 22.

LUTZ, ADOLPH 1886 Zur Morphologie des Mikroörganismus der Lepra. Separat Abdruck aus Dermatologische Studien. Herausg. von Dr. P. G. Unna. Heft. 1, p. 22.

MAASSEN, ALBERT 1905 Ueber Gallertbildungen in den Säften der Zuckerfabriken. Ein Beitrag zur kenntniss der gallertbildenden Bodenbakterien. Arb. a. d. Kaiserl. Gesundheitsamte. Biol. Abt. **5**, 1.

MACE, E. 1897 Traité pratique de Bacteriologie, 3d ed.

MAGGIO (RIVOLTA?) 1887 L'Allevatore, No. 1.

MAGNIN, A. 1878 Les Bacteries. Paris. English translation by G. M. Sternberg. 1880.

MALERBA, P. AND SANNA-SALARIS, G. 1888 Ricerche sul Gliscrobatterio. Rendiconto della R. Accademia delle Science fis. e. nat. di Napoli. Fasc. 6. According to Buchner. Centralbl. f. Bakt. **4**, 486.

MATZUSCHITA, T. 1902 Bakteriologisches Diagnostik.

METSCHNIKOFF, ELIAS 1888 Ueber die phagocytäre Rolle der Tuberkelriesenzellen. Virchow's Arch. **113**, 63–94.

MEYER, ARTHUR 1898 Neues über die Morphologie der Bacterienzelle und die Entwickelungsgeschichte der Bacteriensporen. Sitzungsberichte der Gesellschaft zur Befördung der gesammten Naturwissenschaften zu Marburg, No. 5, p. 49, July 1897.

MIGULA, W. 1894 Ueber ein neues System der Bakterien. Arb. a. d. bakt. Instit. d. Technisch. Hochschule zu Karlsruhe. **1**, 235–238.

MIGULA, W. 1895 Schizomycetes. *In* Engler and Prantl Pflanzenfamilien, p. 20.

MIGULA, W. 1897, 1900 System der Bakterien. Vol. 1, 1897, vol. 2, 1900.

MIGULA, W. 1904 Allgemeine Morphologie. Entwickelungsgeschichte Anatomie und Systematik der Schizomyceten, in Lafar's Handbuch der technischen Mykologie, 2d ed, pp. 1–149.

MILLER, W. D. 1886 Einige gasbildende Spaltpilze des Verdauungstractus, ihr Schicksal im Magen und ihre Reaction auf verschiedene Speisen. Deut. Med. Wochschr. **12**, 117–119.

MIQUEL, M. P. 1879, 1880, 1881 Etudes sur la fermentation ammoniacale et les ferments de l'uree. Ann. de Micrographie. **1**, 414, 417, 506, 552. 1879. **2**, 13, 53, 122, 145, 367, 488. 1880. **3**, 275, 305. 1881.

MORREN, C. F. A. 1830 Bull des Scienc. natur. n. XXVII.

MUELLER, O. F. 1786 Animalcula infusoria et marina, Hauniae.

NAEGELI, CARL WILHELM VON 1842 Botanische Beiträge. Linnaea **16**, 279.

NAEGELI, CARL WILHELM VON 1849 Gattungen einzelliger Algen, physiologisch and systematisch bearbeitet. Zurich.

PERSOON 1822 Mycologia europeae. Sectioprima, p. 96.

PERTY, MAXIMILIAN 1852 Zur Kenntniss kleinster Lebensformen, Bern.

PFEIFFER 1887 Synonymia botanicae, p. 46.

PRAZMOWSKI, ADAM 1880 Untersuchungen über die Entwickelungsgeschichte und Fermentwirkung einiger Bakterien, p. 23, Leipzig.

PRILLIEUX 1879 Sur la nature et la cause de la formation des tubercules qui naissent sur les racines des Legumineuses. Bull Soc. Bot. de France. **26**, 98.

RIVOLTA, SEBASTIANO 1888 Pleurite infettiva nel gatto e nel cane. Giornale di Anatomia Fisiologia e Patologia degli Animali, **20**, 1–8.

ROSENBACH, F. J. 1909 Experimentelle, morphologische und klinische Studien über die krankheitserregenden Mikroörganismen des Schweinerothlaufs, des Erysipeloides und der Mäusesepsis. Ztschr. f. Hyg. **63**, 343–371.

ROTHMANN, E. A. 1904 Glischrobacterium als Ursache der schleimigen Gärung des Menschurins. Centralbl. für. Bakt. I, Abt. Orig., **37**, 491.

SCHNEIDER, A. 1912 Pharmaceutical Bacteriology.

SCHRÖTER, J. 1886 Die Pilze, in Cohn, Kryptogamen-Flora von Schlesien.

SCHRÖTER, J. 1889 Phytomyxinae in Engler and Prantl Nat. Pfl. fam. I, **1**, 7.

SETTE, VINCENZO 1824 Sul l'arrossimento straordinario di alcune sostanze alimentose osservato nella provincia di Padova l'anno 1819. Venezia p. 51.

SMITH, ALLAN J. 1902 Laboratory Exercises in Bacteriology, p. 270.

SMITH, ERWIN F. 1905 Bacteria in relation to plant diseases. Vol. 1. Carnegie Institution, Washington, D. C.

STERNBERG, G. M. 1892 Manual of Bacteriology, N. Y.

TRECUL, A. 1865 Production de plantules amyliferes dans les cellules vegetales pendant la putrefaction. Chlorophylle cristallisee. Compt. rend. hebd. de seances de l'Acad. de Paris. **61**, 432–436.

TREVISAN, V. 1879 Prime linee d'introduzione allo studio dei Batterj italiani. Rendiconti. Reale Instituto Lombardo di Scienze e Lettere IV. Series II, **12**, 133–151.

TREVISAN, V. 1885 Caratteridi alcuni nuovi generi di Batteriacee Atti della Accademia Fisio-Medico-Statistica in Milano. Series 4. **3**, 92–107.

TREVISAN, V. 1887 Se sia vero che il Bacillo imetrofo (Micrococcus prodigiosus di antichi autori) preceda sempre come agente specifico predisponente il calcino del baco da seta. Rendiconti. Reale Istituto Lombardo di Scienze. Lettere. Series II. **20**, 797–804.

TREVISAN, V. 1889 Genera. E sp. delleBatteriacee. According to De Toni, J. B. and Trevisan, V. Schizomycetaceae Naeg. in Saccardo. Sylloge Fungorum **8**, 927.

TROILI-PETERSON, GERDA 1903 Studien über die Mikroörganismen des schwedischen Güterkases. Centralbl. f. Bakt., II Abt., **11**, 138.

VAN LAER, H. 1889 Contributions a l'histoire des ferments des hydrates de carbone. Bacille des kieres tournees. Mem. de l'Acad. royale de Belgique. **43**.

VAN TIEGHEM, PH. 1884 Traité Botanique. Paris, p. 1114.

VUILLEMIN, PAUL 1888 Les tubercules radicaux des legumineuses. Ann. de la Sci. Agron. Franc. et Etrangére. Vme. Ann. T. I., pp. 121–212.

VUILLEMIN, PAUL 1913 Genera Schizomycetum. Annales Mycologici **11**, 526.

WEICHSELBAUM 1887 Centralbl. f. Bakt., **2**, 212.

WINTER, GEORG 1880 Die Pilze, in:—Rabenhorst's Kryptogamen Flora, 2 ed. Vol. 1.

ZIMMERMAN 1880 Review of Bergonzini on Cromobacterium. Bot. Centralbl. **4**, 1528.

ZOPF, W. 1883, 1885 Die Spaltpilze Ed. 1, 1883. Ed. 3, 1885.

A STUDY OF GREEN FLUORESCENT BACTERIA FROM WATER[1]

FRED W. TANNER

From the Laboratories of Bacteriology of the Illinois State Water Survey and of the University of Illinois

Received for publication, December 29, 1916

INTRODUCTION

In all branches of biology a systematic arrangement of the living organisms is essential to good progress. Science has been defined as an exact and systematic statement of knowledge concerning some subject or group of subjects. Such a definition requires that investigations be constantly carried on to incorporate new facts with the old ones into an orderly arrangement. In bacteriology however, the early workers were too busy identifying new forms to give much attention to classification, and when they did turn their attention to this important branch of the science the pleomorphists pointed out the difficulties of systematizing a science the bases of which were constantly changing. It is only in comparatively recent times that the bacteria in water have been given any intensive study of this kind.

HISTORICAL

Classification of water bacteria

The water bacteriologists have been interested primarily in the presence of certain bacteria which are of sanitary significance. They have studied but superficially the large number of other bacteria which may be present. This may have been due

[1] Prepared from a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Bacteriology at the University of Illinois, June, 1916.

to the varied character of water bacteria, for the environment of a water determines its bacterial content, and consequently a water flowing from an unpolluted drainage basin will have a different flora from that of a stream which carries the wastes of domestic and industrial life.

With the exception of some ten bacteria or groups of bacteria, whose presence is supposed to indicate pollution in water, the large number of other bacteria which are present are almost unknown. Information in regard to them would often favor more accurate opinions with regard to quality, and more definite information with regard to the source of water. Certain bacterial standards have been formulated which state that there must not be over 100 bacteria per cubic centimeter in a filter effluent. The character of those bacteria which are present in effluents having a greater number would for instance be important in making deductions with regard to the efficiency of the filters.

The group arrangement has been used for some time in treating the flora of a substance like water. Wyatt Johnston (1894) early called attention to this method. He discussed in his paper the tests which should be used to separate bacteria; and thought that a single strongly marked characteristic was more important for grouping than a number of minor points. The American Public Health Association later appointed a committee (1898) to work out uniform methods for the description of bacteria. This is one of the first instances in which the organized efforts of a society were brought to bear in systematizing methods for the study of bacteria.

Ward (1897) studied the flora of the Thames River and arranged the forms which he isolated in 21 groups, 2 of which are made up of fluorescent bacteria. The separation was often made on very unimportant characteristics. Ward realized this and stated that his work indicated wider limits of the term species than are ordinarily assumed. Fuller and Johnson (1899) in studying the flora of river water made an important contribution to water bacteriology. They divided water bacteria into 13 groups, and used some prominent character for the separation

of each. The fluorescent characteristic played an important rôle. Jordan (1903) made 27 groups when studying 543 strains from the Mississippi, Missouri and Illinois rivers. Two of his groups are; V. *B. fluorescens liquefaciens*, VI. *B. fluorescens non-liquefaciens*. Cornwall (1915) classified water bacteria from a sanitary standpoint. He used, however, morphological data which were too limited to allow a classification of wide application.

This grouping of bacteria introduces the question as to what is a "species." Ward pointed out that we may now be considering varieties of the same organism as separate species. Upon this question there is no agreement among bacteriologists. Winslow (1914) summed up the present conception of this question when he stated that "for practical purposes, however, we must recognized certain types as 'species' or 'varieties' even though they may sometimes intergrade."

The descriptive chart of the Society of American Bacteriologists

Bacteriologists for some time have been working out a system for classifying bacteria. The chart of the Society of American Bacteriologists represents a stage in the development of this effort. A history of the development of the card and of the numerical system of recording characters of bacteria has been prepared by Harding (1910), a very brief outline of which is given here.

Doctor Wyatt Johnston (1894) first called attention to the possibility of using some such method as the Dewey numerical system in the classification of bacteria. A committee was appointed which in its reports suggested the beginnings of such a procedure. Conn (1906) adopted these suggestions in the classification of dairy bacteria. The first real attempt to use such a group number as our present one, reported by Gage and Phelps (1902), was made by Kendall at the Lawrence Experiment Station. The genus classification of bacteria proposed by Migula was used as the basis for their chart.

The present chart has a group number depending on the determination of ten characteristics. It has been recently described by Rahn and Harding (1914). This system of record-

ing the characteristics of bacteria has not met with entire approval on the part of all bacteriologists. One of those who was instrumental in introducing this numerical system has said that it was a thing he regretted. Whether it is, or will be, of any value may be determined after it has been used to study various groups as has already been done in a few cases.

Harding (1910) reported the study of the *Pseudomonas campestris* group. For each strain the same group number was obtained which would indicate that the group number was well fitted to these bacteria. This author believes that it may not "carry the separation to a group synonymous with the ordinary conception of species." The statement seems to have been borne out by later investigations.

Harding and Prucha (1908) used the Society's chart in the study of the flora in cheddar cheese. In their conclusions they state that this method of recording the reaction of cultures is a marked advance in technique and that changes in the cheese flora may be traced more accurately by its use. The sum and substance of their opinion of the chart in its application to the study of cheese bacteria is that it is a valuable means to an end.

Harding, Morse and Jones (1909) in their study of soft rot organisms use the group number in studying their strains. By examining their data it is apparent that the group number is very valuable in studying soft rot organisms, as noted by Harding in 1910 when the same group was studied.

Conn (1906) in his classification of dairy bacteria used a group number, but does not draw any definite conclusions with regard to its value. He still retained names for the organisms which he studied.

More recently, H. J. Conn (1915) has studied 130 cultures of *B. subtilis* by means of the society chart. In selecting these cultures one-half of the determinations represented in the group number were fulfilled because they were implied in the definition of *B. subtilis*. His conclusions are that different group numbers do not always represent different species, and that better methods for making these ten determinations should be devised.

Edson and Carpenter (1912) used the group number in study-

ing bacteria in maple sap. No statements seem to have been made with regard to the value of the Society's chart in the study of the sap bacteria.

The numbers used for recording the various physiological reactions according to the 1912 Society's chart are given in table 1. The numbers of the 1914 chart are identical except those used for action on nitrates.

The fluorescent group of bacteria

This has often been considered a heterogeneous group of bacteria having one property in common—that of excreting a green diffusible pigment capable of causing the medium to fluoresce. Kruse (1896) believes that the members of this group have little phylogenetic relationship, but rather fall into widely related groups. These special bacteria are obviously more conspicuous than the non-fluorescent bacteria, and so have been grouped together. By some bacteriologists however, this group has been called a species.

Ruzicka (1898) believes that there are in the fluorescent group bacteria closely related to the semi-pathogenic organism, *B. pyocyaneus* on the one side and *B. fluorescens-liquefaciens* on the other. Some of his data seem to justify this conclusion.

In Jordan's paper on "The Kinds of Bacteria in River Water" the fluorescent bacteria are discussed. He studied 58 strains of these bacteria, 33 of which liquefied gelatin. The power of liquefying gelatin was found to be closely associated with the ability to coagulate milk. In other characters the two kinds were much alike. All of the strains studied by Jordan were short motile rods. Attention is called to the apparent identity of many of the various forms described in the literature. He mentions the names of about 50 fluorescent bacteria at the close of his paper.

A number of questions may be asked with regard to this group of bacteria characterized by a fluorescent pigment. Is there a good reason other than the formation of this green pigment for setting aside these forms into a group? Is this character of

TABLE 1

Numerical system of recording the salient characters of an organism (group number)

100.0	Endospores produced
200.0	Endospores not produced
10.0	Aerobic (strict)
20.0	Facultative anaerobic
30.0	Anaerobic (strict)
1.0	Gelatin liquefied
2.0	Gelatin not liquefied
0.1	Acid and gas from glucose
0.2	Acid without gas from glucose
0.3	No acid from glucose
0.4	No growth with glucose
0.01	Acid and gas from lactose
0.02	Acid without gas from lactose
0.03	No acid from lactose
0.04	No growth with lactose
0.001	Acid and gas from sucrose
0.002	Acid without gas from sucrose
0.003	No acid from sucrose
0.004	No growth with sucrose
0.0001	Nitrates reduced with evolution of gas
0.0002	Nitrates not reduced
0.0003	Nitrates reduced without gas formation
0.00001	Fluorescent
0.00002	Violet chromogen
0.00003	Blue chromogen
0.00004	Green chromogen
0.00005	Yellow chromogen
0.00006	Orange chromogen
0.00007	Red chromogen
0.00008	Brown chromogen
0.00009	Pink chromogen
0.00000	Non-chromogenic
0.000001	Diastasic action on potato starch, strong
0.000002	Diastasic action on potato starch, feeble
0.000003	Diastasic action on potato starch, absent
0.0000001	Acid and gas from glycerol
0.0000002	Acid without gas from glycerol
0.0000003	No acid from glycerol
0.0000004	No growth with glycerol

The genus according to the system of Migula is given its proper symbol which precedes the number thus:

Bacillus coli (Esch.) Mig. becomes	B 222.111102
Bacillus alkaligenes Petr. becomes	B 212.333102
Pseudomonas campestris becomes	Ps 211.333251
Bacterium suicida (Mig.) becomes	Bact. 222.232203

sufficient importance for such a purpose? Such questions may be answered by a detailed examination of individual strains.

Niederkorn (1900) studied 15 strains of fluorescent bacteria in order to find, if possible, better methods for differentiating the various forms. He thinks that there are only two constant forms, *Bacillus pyocyaneus* (Gessard) and *Bacillus fluorescens-liquefaciens* (Flügge) and that the others are varieties. The work of Eisenberg (1914) shows that the group of fluorescent bacteria is closely related. He reports some work which he did on seven strains and finds intergrading characters which make it difficult to separate them into species and varieties.

Buchanan (1915) has very aptly expressed the condition of bacterial nomenclature. His statement is directly applicable to the fluorescent group. "The naming of bacterial species, genera, and higher groups, indeed the whole subject of bacterial nomenclature, is in a condition which can best be described as chaotic." Many different investigators have studied bacteria which are fluorescent and have given new names to varieties which differed slightly in characteristics. A search of the literature revealed 95 different names which have been given to fluorescent bacteria. There is no reason to think that this number includes all of them. One of the most recent additions to the fluorescent group is *Bacillus cereus* variety *fluorescens* nov. var, which has been reported by Laubach (1916).

Significance of the fluorescent group of bacteria

Bacteria having such a strong aromatic odor may have some significance in the industries. Conn (1910) ascribes certain cases of rancidity in butter to the presence of fluorescent bacteria. The possibility of this is apparent to anyone who has extracted the pigment with any of the usual solvents. Even in small amounts the odor remains for a long time in the room in which such extractions have been made.

Griffon (1909) calls attention to the wide distribution of the fluorescent bacteria in nature, and to the fact that they are present in many plant diseases and cause rot in vegetables.

Edson and Carpenter (1912) in their work, mentioned elsewhere, prove that fluorescent bacteria play an important part in the decomposition of maple sap. Thoni (1911) found fluorescent forms in a bacterial study of lemonade. As has been mentioned before these bacteria are abundant in water supplies. The strains which were studied in this paper were all taken from water supplies in Illinois. Schmelch (1888) found fluorescent bacteria in cold glacial waters. Harrison (1898) found these forms in hail, as did Belli (1902).

Routine analysis of samples of water sent to the Illinois State Water Survey indicate that fluorescent bacteria are rather common in waters from the Illinois and Mississippi rivers. They are especially abundant in the samples from the Illinois river which is rather heavily laden with organic matter, indicating that they are more abundant where organic matter is undergoing decomposition.

Previous studies on the fluorescent group of bacteria

The most important investigation of this group, as such, was carried out at the Vermont Agricultural Experiment Station by Edson and Carpenter (1912). These investigators used the society chart in their study of 42 strains of fluorescent bacteria coming from maple sap which was collected from different orchards in Vermont. The paper contains a complete description of the strains with the following group numbers:

12 strains Pseudomonas	221.2332132	
12 strains Pseudomonas	221.2332133	
7 strains Pseudomonas	221.2322132	
4 strains Pseudomonas	221.2322133	
2 strains Pseudomonas	221.2333133	
1 strain Pseudomonas	221.2323132	
2 strains Pseudomonas	221.2222132	
1 strain Pseudomonas	221.2232133	
1 strain Bacillus	221.2222?32	Nov. Sp.

From these group numbers may be inferred the close relationship of the strains. All of them liquefied gelatin, but it was necessary to keep some of the gelatin tubes longer than six

weeks, the period of time recommended on the Society's chart. Seven strains began to liquefy after three to five months. The time for complete liquefaction was not determined, but at the end of eight and one-half months the tubes when melted and poured into plates, showed growth in pure culture. The group number is made up on the assumption that all strains liquefied gelatin. No spore formers were found in any of the strains.

EXPERIMENTAL

Method of study

The descriptive chart of the Society of American Bacteriologists was used for recording the data in this investigation of the fluorescent bacteria. The group number was determined for each strain under as uniform conditions as possible. One objection to this group number is the lack of uniform and satisfactory methods for the determination of some of the digits. Individual workers may use their own methods, and it is possible that group numbers determined in different laboratories ought not to be compared. It is understood that a code of standard methods is being prepared which ought to allow a wider application of the group number in systematic studies of bacteria.

Media and technique

The media and technique used in this study conformed to Standard Methods for the Examination of Water and Sewage of the American Public Health Association, 1912, except that in place of meat infusion, Liebig's meat extract was used. It was believed that in this way more uniform media were secured.

In those instances where special media were used the composition will be mentioned under the special heading.

Sources of strains

All the strains of fluorescent bacteria were isolated from samples of water which were collected at different places in the

state of Illinois. It was decided to limit this study to the forms obtained from water, and at a later date, if possible, to study another series from a different source. The investigation by Edson and Carpenter was made with strains many of which came from the same sap orchard. In this way their strains may have had some close relationship. The strains which form the basis of the present study are probably in no way related. The source of these strains is given in table 2.

TABLE 2

Surface waters	35
Shallow wells (under 100 feet in depth)	50
Deep wells (over 100 feet in depth)	3
Springs	4
Cisterns	3
Swimming pool	1
Sewage	1
Water of unknown source	1
Water from interstate carrier	1
Ice	1

The colonies were picked from agar and gelatin plates which had been made from water samples received at the laboratory of the Illinois State Water Survey. All of these samples were collected according to instructions which were furnished with the sterile container. They were shipped to the laboratory packed in ice, the examination was immediately started on receipt of the sample and after the plates had been counted for the routine analysis, they were examined for fluorescent bacteria. If none were evident, it was found that they often appeared after the plates had been held at room temperature over night. Young strains were used in all cases. All inoculations into the various media were made either from a twenty-four hour broth culture or from a twenty-four hour agar slant culture.

Description of strains

Since the Society's chart was used in this study the various cultural and morphological characteristics will be mentioned in somewhat the order in which they appear on the chart.

Morphology of strains

All of the strains studied were motile rods, and usually grew in chains. The cells used in studying the morphology were young and were subcultured on plain agar slants before being stained. The vegetative cells were both long and short rods with rounded ends, some of them being distinguished from coccus forms only with great difficulty. The agar slants were incubated at 37°C. for twenty-four hours, and the study of morphology was made from these smears stained with carbol fuchsin.

The cells were measured by means of a Leitz filar micrometer, but the size was not especially significant with these bacteria.

Only four of the cultures showed spore formation. These were 45, 47, 61, and 70. All of the strains possess a great similarity, as is shown by the following group numbers.

45 and 61	121 2332133
47	121 2333133
70	122 2333133

Culture 70 differs from the other three spore formers in not liquefying gelatin. Nitrate reduction is absent in strains 45 and 61. Three of them are from shallow wells, while 47 comes from the Illinois River at Marseilles. A fluorescent spore forming bacillus from water has recently been described by Laubach (1916). The flagella were not studied and he may have been working with a pseudomonas form.

All of the strains were pseudomonads when Loeffler's method was used for staining the flagella. Most of the cultures possessed a *tuft* of flagella on one end, while those with but one flagellum were rare. The length of the flagella was not measured.

Motility was observed with each strain. A few failed to show it upon first observation, but subsequent examinations, however, gave ample proof of motility.

The staining properties were not especially characteristic. All cultures stained with the ordinary aqueous alcohol stains and were Gram-negative.

Capsules were demonstrated on the following cultures by means of Welch's method as described by Muir and Richie (1913).

2, 3 Ps.	221.2223133
6 Ps.	221.2232133
14 Ps.	221.2332133
22 Ps.	221.2222132
26 Ps.	221.2233133
37, 75 Ps.	221.2333133
46 Ps.	221.2332133
50 Ps.	221.2223133
60 Ps.	222.2223132
68 Ps.	222.2233133
79 Ps.	222.2223133

The broth cultures of these strains were invariably viscid. The group numbers are quite similar, but differ slightly in a few characteristics. All but three strains liquefied gelatin. These strains gave slimy growth in most media.

Growth on agar stroke

All strains were grown on this medium and produced a distinct fluorescence. Abundant growth was secured at 37°C. One strain secured from another laboratory refused to grow at 37°, but grew well at 20°. This strain, however, was not included. Strain 100 was peculiar in that the fluorescent color was followed by a permanent red. This may have been due to an acid reaction of the medium.

Growth of potato slants

The potato slants were made in the usual way. In almost every case the growth was spreading and was either moderate or abundant. At the end of about two days incubation it was limited to the line of inoculation, but spread rapidly after longer incubation. The medium was turned to a dark brown or green. Observations were made at the end of ten days, when the potato and the water in the bottom of the tube were poured into a mortar and tested for starch destruction.

Starch destruction

No official method for determining the presence of diastase is available, and each worker uses his own favorite technique. The method used by Edson and Carpenter required the addition of 2 per cent thymol starch paste to a ten day old broth culture. After incubation for eight hours the culture was filtered and tested for reducing sugar by means of Fehling's solution.

The method suggested by Smith was used in this work, as outlined by Harding (1910). Cultures of the bacteria were grown on potato slants for ten days at 37°C. At the end of this time these slants were crushed in a mortar together with the water in the bottom of the culture tubes. This mixture was diluted with distilled water and tested with a weak solution of iodine in potassium iodide for the split products of starch. If the starch mixture had not been sufficiently diluted the blue color which this substance gives with iodine would have entirely masked the color given by some of the decomposition products. The presence of erythrodextrin is shown by a red color. Good results may be obtained with this method after one has become accustomed to the color changes which are involved. Starch agar plates were also used. These were made by adding 1 cc. of a sterile 2 per cent solution of soluble starch to plain agar in Petri dishes. Streaks of the pure culture were made upon this medium and the plates incubated at 37° for five days. At the end of this period a dilute solution of iodine in potassium iodide was permitted to flow over the surface of the plate. With those bacteria which decompose starch a colorless area will be noted about the line of growth. None of the cultures studied were able to decompose starch. This is perhaps not to be expected when one recalls the strong proteolytic action of this group. Over 50 per cent of the cultures were able to break down gelatin, as reported by other investigators.

Agar colonies

Colony growth on agar was always abundant. After the colonies were well developed a large amount of soluble pigment was formed. The edge was always irregular and rarely entire.

Gelatin colonies

All cultures gave abundant growth on this medium. The non-liquefiers grew on the surface and often spread over a large area. Fluorescence was very evident on this medium. The liquefying cultures gave large saucer-shaped colonies which were filled with a flocculent growth.

Gelatin stab

The medium used in this test was made from Gold Label French gelatin. Twelve per cent of gelatin was added to plain peptone bouillon. The medium was put into test tubes to the depth of about 7 cm. Inoculations were made into these tubes by means of a platinum needle, stabbing to the bottom of the test tube. The cultures were incubated for thirty days at 20°C., as recommended on the descriptive chart of the Society of American Bacteriologists.

At the end of thirty days 59 of the cultures had liquefied the gelatin. The gelatin cultures of the other 41 were left in the incubator for over fourteen months. During that time strains 7, 19, 67 and 83 liquefied the medium. With strains 7 and 19, the liquefaction took place at the end of three months. Strains 67 and 83 required about six months for this change. The other 37 strains did not liquefy gelatin in fourteen months when kept at 20°C. The tubes were fitted with rubber caps to prevent evaporation. In determining the group number, only those strains were considered as liquefiers which produced liquefaction in thirty days. In all cases the growth seemed to be best at the surface, although it often extended throughout the tube down into the medium along the line of inoculation. At the end of six or seven days, fluorescence was noticeable. This soon extended throughout the tube, the greatest amount being at the surface.

The characteristic of liquefaction is recognized as an important one in separating bacteria. The possession of a proteolytic enzyme sharply separates a strain from those which do not possess this enzyme. The method for determining this

characteristic must be improved, however, in order that comparable results may be secured by workers from the different laboratories. Diagram I indicates the incidence of gelatin liquefaction, among the strains studied.

Growth in nutrient broth

Good growth was secured with all strains when grown in nutrient broth at 37°C. The medium was practically always cloudy, with a pellicle and sediment. Varying degrees of fluorescence were manifested by the cultures. A few were viscid and strung out when a platinum needle was used. This has been mentioned above under the head of Staining Properties. The growth in nutrient broth was not especially characteristic.

Indol formation

A 1 per cent solution of Witte's peptone was used in this test. The tubes were inoculated from a twenty-four-hour broth culture and were incubated at 37°C. for ten days. At the end of this incubation period, 1 cc. of paradimethylamidobenzaldehyde was added to the culture tube. None of the cultures formed indol. With the strains which produced a large amount of fluorescent pigment a red color was very often secured, but this was not due to indol since the culture tubes did not have the characteristic odor of putrefaction. This red color may have been due to the pigment.

Formation of hydrogen sulfide

To study this characteristic, a special medium prepared by Redfield (1912) was used. This was made by adding 300 grams of Witte's peptone and 75 grams of potassium chloride to 700 cc. of boiling tap water. This mixture was heated to dissolve as much peptone as possible, and the solution was then cooled and diluted to 1 liter. It was again boiled, plugged with cotton and allowed to stand in an ice box for at least twenty-four hours. At the end of this time the medium was filtered and transferred to special flasks used in the routine analysis of water.

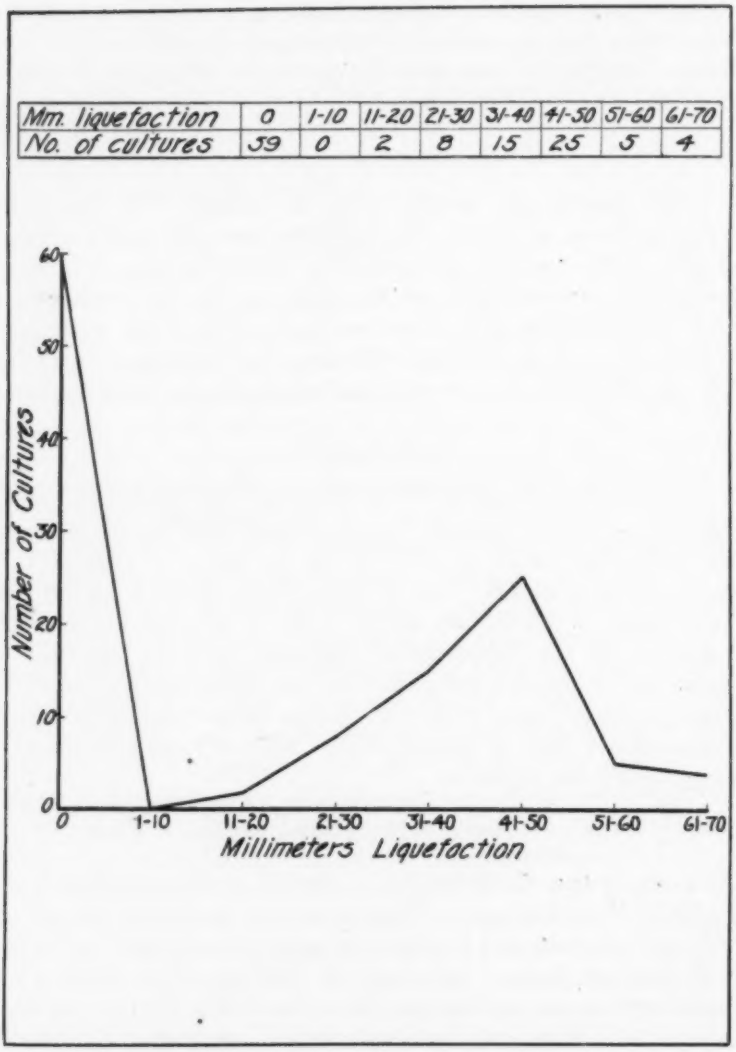


DIAGRAM I. CURVE INDICATING INCIDENCE OF GELATIN LIQUEFACTION BY FLUORESCENT BACTERIA

The method here used was a modification of Redfield's, because the flasks used by him in routine water analysis could not readily be adapted to the study of pure cultures. In his method, 10 cc. of his special medium were diluted to 100 cc. in a special flask with 90 cc. of the sample under investigation. This flask was fitted with a ground glass stopper which held the lead acetate paper. In order to adapt this medium to the study of pure cultures, it was made up one-tenth as strong as given above and put into a test tube fitted with a one-hole rubber stopper carrying a piece of glass tubing. A strip of lead acetate paper was held in this tube by means of cotton. Tin foil was twisted about the end of the glass tube. After inoculation from a twenty-four hour culture the tubes were incubated at 37°C. for thirty days. At the end of this period darkening of the paper indicated hydrogen sulfide formation.

Some of the strains studied in this series produced large amounts of hydrogen sulfide. Strains 12, 19, 23, 30, 43, 45, 47, 48, and 52, produced sufficient hydrogen sulfide at 37°C. in much less time than thirty days to darken 2 cm. of a strip of paper 25 by 2 mm. Forty-six of the cultures produced hydrogen sulfide from Redfield's medium.

Redfield has collected the names of the bacteria which have been reported to produce hydrogen sulfide. The following fluorescent bacteria are included.

In two days, *Bacillus fluorescens-non-liquefaciens;* in three days, *Bacillus pyocyaneus;* in ten days, *Bacillus fluorescens-liquefaciens;* in thirty days, *Bacillus cyanogenus* (Fluorescent?); in varying lengths of time, *Bacterium immobile, Pseudomonas fluorescens* (Flügge), *Pseudomonas pilocyanea.*

Edson and Carpenter secured hydrogen sulfide formation in 20 of the 42 cultures which were isolated from maple sap. This gives a percentage of approximately 48, while that obtained with our cultures from water was 46 per cent. The method of Edson and Carpenter consisted in suspending a strip of lead acetate paper above an ordinary broth culture. From these two investigations it would seem that plain broth was about as efficient as Redfield's medium for observing the formation of

hydrogen sulfide by bacteria. In a paper by Chamot and Redfield (1915) on the same subject the statement is made that hydrogen sulfide is more rapidly produced in a mixed culture than in a pure culture. The peptone is probably split to abiuret compounds among which are cysteine and cystine. From these it is easy to imagine how H_2S may be split off. This reaction has been studied by Sasaki and Otsuka (1912), Burger (1914), and Tanner (1917).

Action on carbohydrates

No gas was formed by any of the strains. The reaction in sugar broths was determined after two days' incubation at 37°C. Five cubic centimeters of the media were diluted with 50 cc. of distilled water and titrated, after boiling, with $\frac{N}{10}$ NaOH. Rogers and Davis (1912) commenting on the value of such data for classification work state,

> Mention has already been made of the objections to the use of fermentation of sugars and similar substances. The question of the constancy of these reactions has been the subject of investigation, and while there is some disagreement of opinion among those who have studied the question most carefully it seems that they are at least as constant as any of the characters ordinarily used in classification.

In this study phenolphthalein was used as the indicator, as advised on the 1912 chart of the Society of American Bacteriologists. The ideal method would have been the determination of the true acidity by means of the hydrogen electrode, as has been pointed out by the recent work of Clark and his coworkers.

The rectangular polygons as shown in Diagram II indicate the distribution curves for the various strains with regard to action on glucose, sucrose, lactose and glycerol.

With regard to glucose, the curve shows a single mode which indicates one type of fermentative action. None of the strains formed alkali. The distribution curve for the action on sucrose shows a single mode and one single type of bacteria. All of these bacteria are probably non-sucrose fermenters. A single

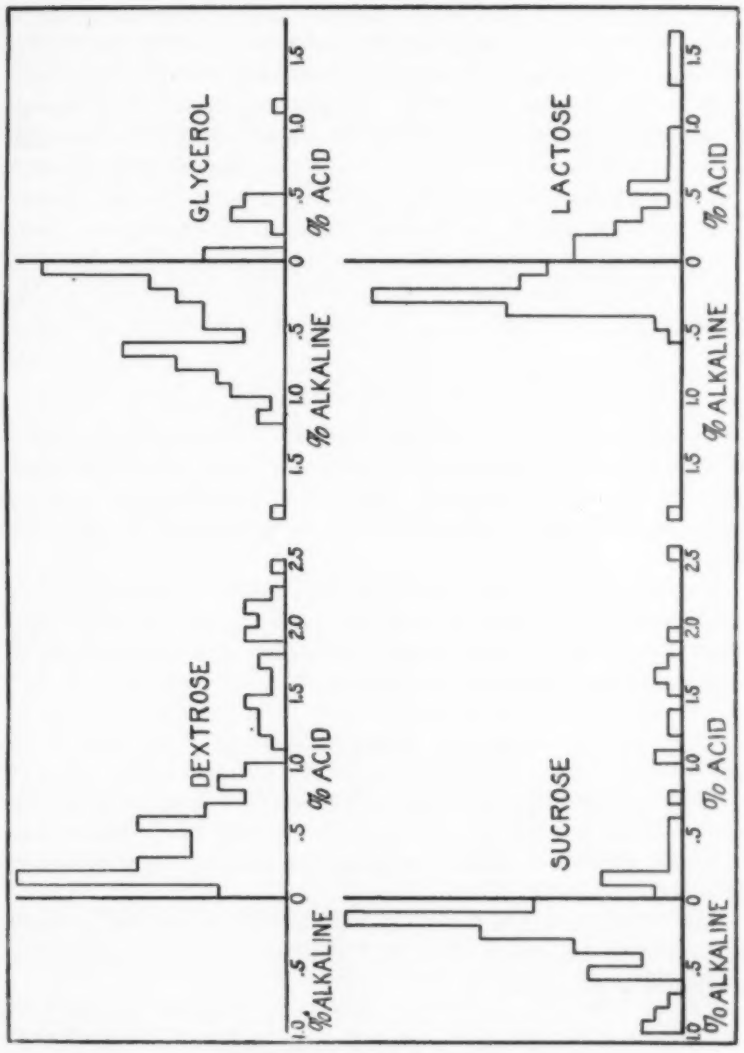


DIAGRAM II. INCIDENCE OF FERMENTATION OF CARBOHYDRATES AND GLYCEROL BY FLUORESCENT BACTERIA

mode is also secured in the distribution curve for lactose. Here, again, a single type of bacteria is indicated. With regard to the action on glycerol, however, two modes are evident which may indicate two types of bacteria. In general, these 100 strains of fluorescent bacteria are alike with regard to fermentation of glucose and non-fermentation of sucrose, lactose and glycerol. Edson and Carpenter (1912) state that with the fluorescent bacteria from maple sap no sharp line of differentiation exists between acid production and no acid production. The distribution curve for the fluorescent bacteria from water indicate the same thing with regard to sucrose, lactose and possibly glycerol.

Growth in Uschinsky's medium

This medium was made up after the following formula and sterilized in the Arnold on three successive days: Distilled water, 1000 cc.; glycerol, 35 grams; sodium chloride, 6 grams; calcium chloride, 0.1 gram; magnesium sulfate, 0.35 gram; dipotassium phosphate, 2 grams.

Vigorous growth was secured in this medium with all strains except no. 10. In many cases the medium was strongly fluorescent. A pellicle was formed by many strains and many of them exhibited a very viscid growth.

Growth in Frankel's medium

This medium was prepared after the following formula: sodium chloride, 5 grams; monocalcium phosphate, 2 grams; ammonium lactate, 6 grams; asparagin, 4 grams; distilled water, 1000 cc.; N NaOH, 20 cc.

The medium was filtered and sterilized in the Arnold sterilizer on three successive days All strains grew abundantly and produced much pigment.

Growth in Sullivan's medium

This medium (Sullivan (1905)) which was found to support good pigment formation was made up as follows: distilled water,

1000 cc.; asparagin, 1 per cent; magnesium sulfate, 0.20 per cent; dipotassium phosphate, 0.10 per cent.

Growth in it was abundant but did not reach a maximum as quickly as in Frankel's medium. None of these strains could reduce the magnesium sulfate to hydrogen sulfide as determined by the method described.

Oxygen relations

All strains were found to be facultative anaerobes. The determination was carried out in a vacuum desiccator in the bottom of which were placed about 50 grams of pyrogallol. The cultures were made on agar slants and placed in the desiccator; and by means of a vacuum pump the pressure was reduced as far as possible under these conditions. After this was done a strong solution of KOH was allowed to be sucked into the bottom of the desiccator by slightly opening the glass stop cock. By this technique it is believed that a minimum supply of oxygen was left.

The desiccator was incubated for five days at room temperature, which was about 25°C. At the end of that time there was visible growth. It was very restricted but quite apparent. Some doubt was felt with regard to the results, since these bacteria have always been described as strict aerobes, but since growth was quite visible, the group number is determined on the ground that they are facultative anaerobes and are able to carry on a limited activity under reduced supplies of oxygen.

Growth in plain milk

This medium was made by thoroughly mixing 100 grams of Merrell-Soule skim milk powder in 1000 cc. of distilled water. It was then beaten with an egg beater, filtered and sterilized in 75 cc. quantities in Erlenmeyer flasks. Inoculations were made from twenty-four hour cultures and the flasks incubated at 37°. The fluorescent bacteria seem to arrange themselves into four general groups with regard to their action on milk.

One group is made up of those cultures which produce prompt

coagulation in two days. Strains 1, 5, 13, 16, 17, 18, 23, 24, 30, 31, 39, 57, and 60, coagulated the milk in this time. The curd formed was solid in consistency and in many cases settled to the bottom of the flask. When peptonization took place this curd was decomposed until the whole flask had assumed a golden color.

Another group produced clearing without coagulation. The rapidity of this action varied. With some of the strains it was evident after twenty-four hours' incubation at 37°. Clearing usually started at the surface—a dark transparent layer being formed which rapidly extended downward. With a few of the strains, however, the entire contents of the flask seemed to change to a thinner consistency. The final product was of a golden color. The green pigment which was often produced in large amounts in this medium, combined with this golden color, to give the solution a striking appearance.

The third type was made up of those strains which seemed to have no effect on the milk in twenty days. It is possible that some change might have been secured, had these cultures been held longer.

The fourth group consisted of those strains which rendered the medium slimy. This was very apparent after twenty days. The strains which possessed capsules always produced a slimy growth in broth and milk.

The Society's chart calls for the determination of the reaction at the end of one, two, four, ten, and twenty days. This was attempted, but erratic results were obtained, since the medium was so highly colored that it was impossible to determine the real end point with phenolphthalein. The green pigment seemed to be the greatest factor in masking the neutral point. Another possible reason why varying results were obtained is that phenolphthalein is not sensitive in solutions which contain ammonia; and much ammonia is formed in the splitting of casein. Were the solutions boiled to remove carbon dioxide, some of the volatile compounds might be driven off and cause a greater error than the carbon dioxide itself. For determining the group number the reaction at the end of the tenth day

was taken. By that time a constant reaction had been established, and continued incubation simply increased it in the same direction.

Growth in litmus milk

This medium was prepared after the same method that was used in the preparation of plain milk. Kahlbaum's azolitmin was used as the indicator. The milk was inoculated from a twenty-four-hour broth culture by means of a platinum loop, and incubated at 37°C. for twenty-five days. A detailed account of all the changes in this medium will not be included. In many cases the tubes turned alkaline at the surface and the blue color gradually extended downward until the whole tube was changed. When peptonization occurred this continued until the whole tube had turned to an orange color.

Many of the strains possessed no proteolytic enzyme and thus produced no change in plain milk. Some of the strains formed alkali which soon gave way to a permanent acid. In many cases the curdling of the milk could not be entirely explained by the concentration of the acid. It was doubtless due to the presence of a rennin-like enzyme.

Peptonization of casein

With most of the strains casein peptonization was apparent in the flasks used in the titration of the acidity of milk, but with the others it was impossible to tell whether the casein was attacked. In order to determine this character, Hasting's (1904) milk agar was used. This was prepared by cooling a tube of melted agar and adding to it 1 cc. of sterile skimmed milk. This was then poured into a sterile Petri dish and thoroughly stirred. When solidified, it was inoculated by making streaks on the surface, after which the plates were incubated for five days at 37°C. A clear zone appeared around the line of inoculation on those plates which were inoculated with the strains capable of peptonizing the casein. To verify this conclusion, dilute acetic acid was added to the plate, and if the zone remained clear the strain was recorded as one which would peptonize.

Forty-two strains were able to decompose casein. This reaction, of course, was more marked with a few of the forms than with the majority.

Temperature relations

The temperature relations of any group of bacteria are very important. It is often believed that since an organism is found in nature outside the body, it has its optimum temperature and grows best at temperatures near 20°C., and that growth is restricted at 37°. The literature on fluorescent bacteria indicates that very few of these forms refuse to grow at 37°. All the cultures used in this study were found to grow well at this temperature. The amount of change brought about by bacteria may be said, generally speaking, to be a function of the incubation temperature and period of incubation, in accordance with the laws which govern enzyme action and the relation of temperature thereto.

Fluorescence

This characteristic was a necessary requisite for the inclusion of a culture in the investigation. It is closely related to the pigment which these bacteria form. Some bacteriologists have attempted to separate *B. pyocyaneus* and *B. fluorescens-liquefaciens* on the ground that *B. pyocyaneus* possessed no fluorescent pigment. This separation is probably more apparent than real. There were all gradations among the cultures of this series with regard to this characteristic. The forms which liquefied gelatin and casein produced more fluorescent pigment than those which did not break up these two compounds.

Color in nature

The theories with regard to color or pigment formation are reviewed by Sullivan (1905). He divides pigments into two divisions, viz: structural and pigmental. Pigments in nature are divided into: (a) Pigments of direct importance, as in respiration; (b) derivatives of such pigments; (c) waste products,

or derivatives of such; (d) introduced pigments; (e) reserve pigments or pigments associated with reserves.

Sullivan also reports Beyerinck's (1891) division of chromogenic bacteria, which is as follows: (a) Chromophorous bacteria —forms in which the pigment serves some purpose in the cell, as chlorophyll; (b) chromoparous bacteria—forms which excrete the pigment as a waste substance; and (c) parachrome bacteria —forms which retain the pigment in their cells.

The subject of bacterial pigments has been much discussed; but only a few of the publications will be mentioned here. Wasserzug (1887) studied *B. pyocyaneus* or the organism of green pus and found that in the same cultures not all the cells produced the same pigment. To avoid this variation as far as possible, he worked with cultures which had been rejuvenated by successive transfers and inoculations into rabbits. Wasserzug states

> Il semble qu'on puisse distinguer deux periodes dans la vie de l'organisme coloré: dans la premiere il prefers et accommoda a ses besoins son milieu de culture; dans la second, il produit et secrete, la matière colorante.

He tried the effects of antiseptics and found that the points where pigment formation and growth stopped differed but slightly. This would seem to indicate that the colored pigment was merely a waste produce resulting from cellular metabolism.

Jordan (1899) studied the conditions under which the diffusible fluorescent pigment was produced. He used synthetic media and concluded that the presence of phosphorus and sulfur was essential to vigorous fluorescing properties. He added that the fluorescent property might be of no benefit to the cell.

Babes (1889) studied the colored materials from *B. pyocyaneus*. To secure his pigments he inoculated neutral peptone gelatin with a strain isolated from an abscess. The medium was inoculated and left for six weeks at room temperature. The color varied and an odor of linden flowers was noticeable. Babes used solvents and distillation methods to remove his pigment,

and probably secured "the pyocyanin" of Fordos (1863). This was blue in alkaline and red in acid solution, and crystallized in rhombic crystals. A red-brown substance was also secured which was greenish in refracted light, soluble in water and insoluble in chloroform. In acid solution fluorescence was lost, but was acquired again in alkaline solution. By distillation a colorless substance with a peculiar odor was obtained. This differed from the original and was supposed to be a decomposition product.

Thumm (1905) found that all species produced the same pigment, which differs from the reports of other investigators. He was unable to confirm the findings of others that several pigments were produced. He found that this group was made up of vigorous alkali formers, and that glucose was fermented to acids which were neutralized later by the formation of ammonia.

Boland (1899) believes that two pigments are formed, a fluorescent one, which is formed by many other bacteria, and pyocyanin which changes into a red brown pigment by oxidation.

Krause (1900) studied the symbiosis of *B. pyocyaneus* with pus formers and found that the aromatic odor was almost always present, and that as long as *B. pyocyaneus* predominated over the pus formers, the green color did not appear. When *B. pyocyaneus* cells were removed from the culture, they again formed the green pigment. Certain gases were tried, and with hydrogen good growth was secured but no pigment. With CO_2 and the vacuum method no growth was secured. In his study of the pigments the following were secured:

(1) Water extract, greenish yellow fluorescence; (2) 80 per cent alcohol, yellowish green fluorescence; (3) glycerol, blue-green fluorescence; (4) Amyl alcohol, grass green fluorescence; (5) chloroform, blue; (6) ether, yellow blue.

Nogier, Dufourt and Dujol (1913) studied a strain from a lesion and found a red pigment of the color of vinegar along with the fluorescent and red-brown pigment usually described. This red pigment was formed in glycerol broth on the fourth day. Acid and alkali seemed to suppress pigmentation, as did pure oxygen.

Gessard (1890) believed that two pigments were formed, a fluorescent green and pyocyanin.

Sullivan (1905) finds that pyocyanin formation is independent of the presence of phosphate or sulfate. He did find, however, that phosphorus and sulfur were essential. He recognizes a fluorescent pigment and pyocyanin and states that the same variety of *B. pyocyaneus* may be made to display either of these functions or both. Sullivan believes that "the production of pigment is not an essential vital act. As it is of no discoverable advantage to the organism possessing the power of producing it, its production is purely accidental."

In a study of this pigment by the writer, a 4 liter flask of plain broth was inoculated with strain 37 and left for eight weeks at a temperature of about 25°C. At the end of that time the medium had assumed a dirty green color with a heavy precipitate in the bottom of the flask. When the flask of broth was tested for growth, a large number of living bacteria were found. This culture was filtered through paper into a large bottle from which different portions were taken for study.

About 1½ liters were precipitated with lead acetate and allowed to stand over night. In the morning this material was filtered and divided into three portions. Each of these was extracted with ether, chloroform and ligroin. The chloroform was the only solvent which removed any of the pigment. It removed a blue pigment which was increased in amount by subsequent shakings in a separatory funnel. In four or five days this blue chloroform solution changed to a red when left in bright light. No reagents seemed able to change it back to the green color. In the dark the blue chloroform solution changed from a deep blue color to a dark green which was permanent.

Some of the chloroform solution was allowed to evaporate in a crystallizing dish. A black residue which possessed crystalline structure was left and this had a very strong odor similar to some of the aromatic ammonium bases. This substance was soluble in alcohol and water, insoluble in ether and red in acid solution. Neutralization restored the green color again.

The work here reported agrees with that reported by the

early authors. Ledderhose (1888) believes that this pigment which has been called pyocyanin belongs to a group of aromatic substances closely related to the anthracene group. It was thought that this pigment might be related to anthocyanin which is supposed to have some relation to the flavon or xanthon groups. This may exist in the form of a colorless glucoside in the plant and be capable of oxidation only after it has been liberated. Repeated tests by Molisch's reaction failed to demonstrate its glucosidal character either before or after hydrolysis. More work on this subject is planned.

Reduction of nitrates

Nitrate reduction tests were made on media containing distilled water, 1000 cc.; Witte's peptone, 1 gram; and potassium nitrate, 2 grams.

Thirty cubic centimeters of this medium were sterilized in small Erlenmeyer flasks. These were inoculated from a twenty-four-hour broth culture and incubated for five days at 37°C. At the end of this period 1 cc. of the nitrate broth culture was removed by a sterile pipette and diluted to 50 cc. in a Nessler tube with nitrite free water.

The method used for determining nitrites was that usually employed in water laboratories, and it is believed that it is not too delicate for bacterial work if blank determinations are made. To each of the Nessler tubes prepared above was added 1 cc. of an acid solution of naphthylamine hydrochloride and 1 cc. of a saturated solution of sulfanilic acid. The tubes were allowed to stand for thirty minutes in the colorimeter before being examined. Control tests were always made at the same time.

Fifty-one of the strains reduced potassium nitrate when incubated for five days at 37°C.

Formation of ammonia

The nitrate broth cultures were used for this purpose. At the end of ten days 2 cc. of this broth culture were withdrawn and diluted to 50 cc. with ammonia free water. Nessler's

reagent was added to the tubes and control tubes, and comparison made in a colorimeter used for determining ammonia in water analysis.

Fifty-two of the 100 cultures produced ammonia in ten days at 37°C. Plain nutrient broth cultures were often tested for ammonia production, and all strains were found to produce ammonia. The plain broth cultures were alkaline to phenolphthalein.

Vitality of fluorescent bacteria

The members of this group seem to be able to resist very unfavorable conditions. No special experiments have been made, but much evidence from a number of sources is available.

Broth suspensions of *B. pyocyaneus* were suspended in the Urbana septic tank and daily counts made. The number of bacteria increased regularly until the end of the period, which indicates that this bacterium is able to live in such an environment.

It has been noticed that tubes of supposedly sterile culture media which spoiled were often infected with bacteria of this group. Thus it is apparent that these bacteria are able to resist high temperatures for short periods. It is well known among physicians that once a hospital is infected with *B. pyocyaneus*, it is disinfected with great difficulty.

Cultures of these bacteria live for a long time on laboratory media. Agar slants which have been allowed to dry for six months at room temperature were found to support living fluorescent bacteria. Cultures of strains 22 and 37 were left for a year and a half with infrequent transfers and in each case good growth was secured from the old cultures. These cultures did however lose some of their pigment-producing property.

Rettger and Sherrick (1911) report an example of resistance by a member of this group. They state that an old culture of *B. pyocyaneus* began to lose its property of producing the green pigment. An agar slant of this bacterium was placed on the top of an incubator in April and left until October. When examined at this time the agar was dried to a hard mass and it was thought unlikely that any living bacteria were present.

early authors. Ledderhose (1888) believes that this pigment which has been called pyocyanin belongs to a group of aromatic substances closely related to the anthracene group. It was thought that this pigment might be related to anthocyanin which is supposed to have some relation to the flavon or xanthon groups. This may exist in the form of a colorless glucoside in the plant and be capable of oxidation only after it has been liberated. Repeated tests by Molisch's reaction failed to demonstrate its glucosidal character either before or after hydrolysis. More work on this subject is planned.

Reduction of nitrates

Nitrate reduction tests were made on media containing distilled water, 1000 cc.; Witte's peptone, 1 gram; and potassium nitrate, 2 grams.

Thirty cubic centimeters of this medium were sterilized in small Erlenmeyer flasks. These were inoculated from a twenty-four-hour broth culture and incubated for five days at 37°C. At the end of this period 1 cc. of the nitrate broth culture was removed by a sterile pipette and diluted to 50 cc. in a Nessler tube with nitrite free water.

The method used for determining nitrites was that usually employed in water laboratories, and it is believed that it is not too delicate for bacterial work if blank determinations are made. To each of the Nessler tubes prepared above was added 1 cc. of an acid solution of naphthylamine hydrochloride and 1 cc. of a saturated solution of sulfanilic acid. The tubes were allowed to stand for thirty minutes in the colorimeter before being examined. Control tests were always made at the same time.

Fifty-one of the strains reduced potassium nitrate when incubated for five days at 37°C.

Formation of ammonia

The nitrate broth cultures were used for this purpose. At the end of ten days 2 cc. of this broth culture were withdrawn and diluted to 50 cc. with ammonia free water. Nessler's

reagent was added to the tubes and control tubes, and comparison made in a colorimeter used for determining ammonia in water analysis.

Fifty-two of the 100 cultures produced ammonia in ten days at 37°C. Plain nutrient broth cultures were often tested for ammonia production, and all strains were found to produce ammonia. The plain broth cultures were alkaline to phenolphthalein.

Vitality of fluorescent bacteria

The members of this group seem to be able to resist very unfavorable conditions. No special experiments have been made, but much evidence from a number of sources is available.

Broth suspensions of *B. pyocyaneus* were suspended in the Urbana septic tank and daily counts made. The number of bacteria increased regularly until the end of the period, which indicates that this bacterium is able to live in such an environment.

It has been noticed that tubes of supposedly sterile culture media which spoiled were often infected with bacteria of this group. Thus it is apparent that these bacteria are able to resist high temperatures for short periods. It is well known among physicians that once a hospital is infected with *B. pyocyaneus*, it is disinfected with great difficulty.

Cultures of these bacteria live for a long time on laboratory media. Agar slants which have been allowed to dry for six months at room temperature were found to support living fluorescent bacteria. Cultures of strains 22 and 37 were left for a year and a half with infrequent transfers and in each case good growth was secured from the old cultures. These cultures did however lose some of their pigment-producing property.

Rettger and Sherrick (1911) report an example of resistance by a member of this group. They state that an old culture of *B. pyocyaneus* began to lose its property of producing the green pigment. An agar slant of this bacterium was placed on the top of an incubator in April and left until October. When examined at this time the agar was dried to a hard mass and it was thought unlikely that any living bacteria were present.

Transfer to fresh media, however, gave good growth and much pigment was produced.

Burge and Neill (1915), in a paper on the comparative resistance of fluorescent and non-fluorescent bacteria to ultra-violet light, conclude that fluorescent bacteria are better able to resist ultra-violet light than those which do not produce this pigment. Their cultures of fluorescent bacteria were secured from among those which form the basis of this investigation. Table 3 indicates the results which they secured with the fluorescent bacteria. The numbers in parentheses are those which the cultures bear in this paper; the other numbers are those which Burge and Neill assigned to them.

TABLE 3

Germicidal action of ultra violet light on fluorescent bacteria

TIME OF EXPOSURE	NUMBERS OF BACTERIA PER CUBIC CENTIMETER						
	1 (2)	2 (3)	3 (13)	4 (15)	5 (22)	6 (25)	7 (37)
seconds							
0	72 M*	80 M	28 M	70 M	75 M	33 M	25 M
20							
40							
60	750	813	55	210	460	12	88
80	459	623	29	79	211	10	20
100	223	221	25	56	113	7	14
120	128	102	20	29	103	5	12
140	97	88	15	18	56	4	8
160	81	73	12	13	38	3	2
180	43	29	10	6	25	3	1
200	31	15	3	3	11	2	1

* Million.

This table indicates that these bacteria have a high resistance to ultra-violet light. In order to secure some basis for comparing this character, the same authors exposed such common forms as *B. coli* (*communis*), *Pseudomonas violacea*, *Sarcina lutea* and *B.* (*proteus*) *vulgaris*, which are not fluorescent, to the effects of the ultra-violet light. In no case were any of these bacteria able to resist the effects of the ultra-violet light for more than 200 seconds. Most of them could not survive an exposure of

160 seconds. With the fluorescent bacteria, however, the results are quite different. In each case a few cells were found alive at the end of 200 seconds. Curves accompany the paper by these investigators which indicate that the death rate follows the monomolecular law. Burge and Neill explain this resistance of fluorescent bacteria to ultra-violet light by assuming that "the fluorescent bacteria protect themselves from the coagulating effect of the ultra-violet light by converting the short wave lengths to longer waves and hence disposing of energy of the absorbed short waves." Non-fluorescent bacteria were unable to do this.

Divisions of strains into groups

The group numbers allow the following separation of strains.

2	121.2332133	61, 45
1	121.2333133	47
1	122.2333133	70
2	221.2222132	22, 15
1	221.2222133	13
2	221.2223132	23, 20
6	221.2223133	24, 17, 2, 50, 31, 3
4	221.2232133	34, 6, 88, 86
4	221.2233133	71, 29, 26, 25
1	221.2322132	27
1	221.2323132	30
1	221.2323133	48
2	221.2332132	62, 59
14	221.2332133	99, 91, 94, 84, 85, 73, 46, 28, 21, 16, 87, 14, 10, 74
2	221.2333132	69, 66
12	221.2333133	98, 90, 75, 72, 67, 64, 63, 37, 35, 32, 12, 65
1	222.2222133	19
1	222.2223132	60
6	222.2223133	93, 83, 80, 79, 78, 33
1	222.2232133	95
1	222.2233132	82
6	222.2233133	92, 68, 81, 52, 96, 89
1	222.2322132	5
1	222.2323133	100
3	222.2333132	39, 57, 55
10	222.2333133	97 77, 76, 56, 54, 53, 43, 9, 8, 7
13	222.2332133	58, 51, 49, 44, 42, 41, 40, 38, 36, 18, 11, 4, 1

Twenty-seven groups are thus secured, many of which are separated by but one characteristic from those closely related to them. With the group number as it now stands, it is possible to obtain 276,480 different types of bacteria.

Diagram III presents a brief characterization of the action of these cultures on some of the more important substances. Such a diagram was first used by Rogers and Clark (1912).

Neglecting nitrate reduction and gelatin liquefaction as variable characters of little value for classification, the following nine groups are obtained:

	Strains
22 ± . 233 ± 133	49
22 ± . 223 ± 133	15
22 ± . 222 ± 133	14
22 ± . 233 ± 132	7
22 ± . 222 ± 132	5
12 ± . 233 ± 133	4
22 ± . 232 ± 132	3
22 ± . 223 ± 132	1
22 ± . 232 ± 133	2

Inspection of the above numbers indicates a very close relationship. Barring the four spore formers, there is an apparent intergrading of characters. Were all of the strains strictly considered as non-fermenters of lactose and sucrose, as might be inferred from Diagram II, the number of groups would be further reduced to five. They might be regarded as falling into two groups with regard to glycerol, and this characteristic might be regarded as a basis for separation.

DISCUSSION OF RESULTS

A comparative study of 100 strains of fluorescent bacteria from water by means of the group number system as expressed on the descriptive chart of the Society of American Bacteriologists, 1912, places them in 27 groups. Twelve of these are made up of but one strain and are separated from the adjacent groups by but one characteristic. If gelatin liquefaction and nitrate reduction are excluded as variable characteristics for classification purposes, as has been suggested by different investigators,

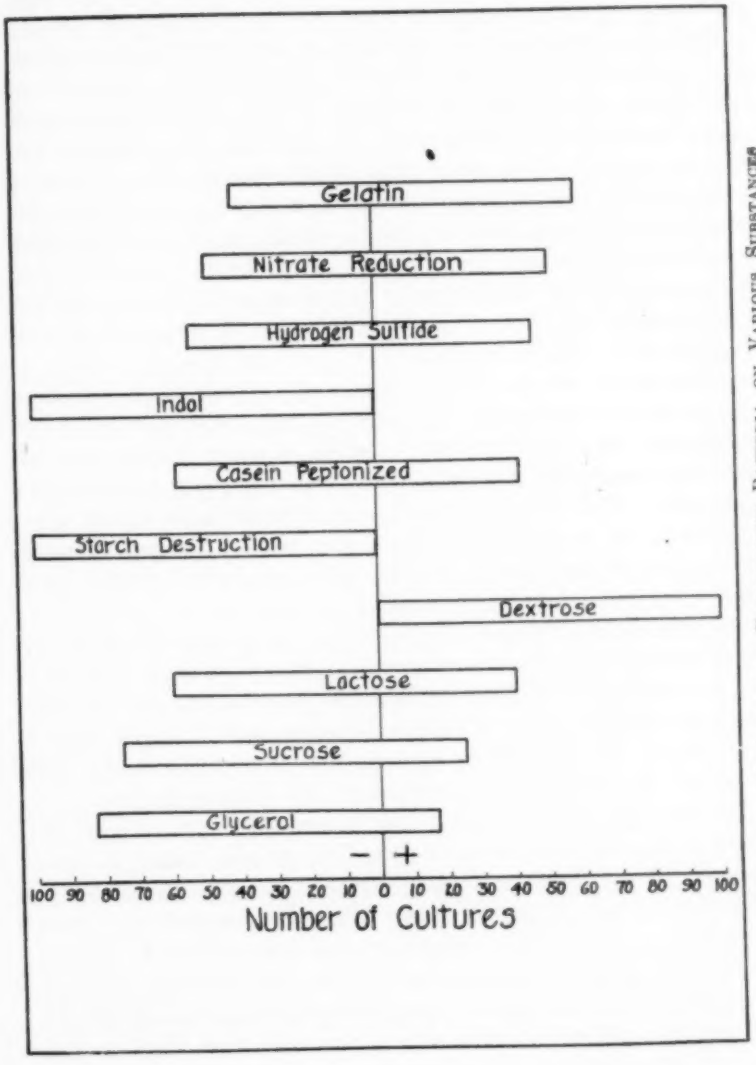


DIAGRAM III. ACTION OF FLUORESCENT BACTERIA ON VARIOUS SUBSTANCES

the 100 strains fall into 9 groups all of which are very closely related.

The group number probably does not separate bacteria along natural lines, but it does constitute a convenient method for considering the characters of such closely related bacteria as make up the fluorescent group. It obviates the necessity for bacterial names which have very little meaning in present bacterial work. Especially true is this in regard to the fluorescent group. The group number places equal emphasis on all the determinations which it expresses. It is quite probable, too, that many of the inadequacies of the group number may be explained by the lack of proper methods for determining the characters for which it calls.

Four of the cultures form endospores, which have been accepted by bacteriologists as a reliable and important basis for separating bacteria. De Bary placed so much importance on this that he described minutely the formation and germination of spores. This characteristic is one of importance which indicates that the fluorescent bacteria may not be a genetic group. Edson and Carpenter, however, report no spore-forming fluorescent bacteria in their study of maple sap.

The presence of a proteolytic enzyme for gelatin might be of more value in classification were a satisfactory method available for detemining its presence. With the fluorescent bacteria this has been taken as the sole difference between certain members of the group. The 100 strains forming the basis of this study were about evenly divided with respect to this characteristic.

Gelatin liquefaction paralleled casein digestion closely, although sixteen strains which liquefied gelatin failed to split casein. Of the cultures which liquefied gelatin, the majority liquefied between 40 and 50 mm. The others graded away from this group and one may infer that the determination, as it is now made, is not sufficiently delicate for classification purposes. Those strains which required four or five months for liquefaction to appear might be regarded as the links between the liquefiers and the non-liquefiers, or they might represent transitional forms which are in the act of acquiring or losing this characteristic.

Investigations with regard to better technique for determining gelatin liquefaction are under investigation in this laboratory.

The strains are all reported as facultative anaerobes. This is another determination called for in the group number for which there is no satisfactory technique. Formerly much importance was attributed to growth along the line of inoculation in stab cultures. This is beset with too many objections. A medium itself may contain sufficient dissolved oxygen to support growth. Evidence of growth in the closed arm of the fermentation tube has also been used to determine anaerobiosis. This limits the determination to the particular substance from which the oxygen was taken. A standard technique for this determination would have much significance.

With regard to diastatic action on starch, the personal equation is given too much importance in recording this characteristic on the Society's chart.

All of these cultures correlate with regard to fermentation of glucose, production of fluorescent pigment, absence of diastasic action on starch, negative indol formation, and probably non-fermentation of sucrose and lactose. The modes in the curve of Diagram II indicate that there is probably one type of action on lactose and sucrose and possibly two types with regard to glycerol. However, with glycerol one of these modes is quite near the other and close to the line where experimental error might connect it more evidently with the other. Since these characters agree for so many cultures, all of which are from widely separated sources, they are probably of significance. The absence of diastatic action presupposes no amylase and correlates well with the action on the other carbohydrates, lactose and sucrose. As stated before, it is recognized that the use of phenolphthalein as the indicator in determining reactions is arbitrary, and that determination of true acidity by means of the hydrogen electrode may prove that all of these strains were unable to ferment carbohydrates. Negative indol formation may indicate that peptone is not split to amino acids and other products including tryptophan which is the precursor of indol.

The explanation of the correlation between acid formation in

glucose broth and production of fluorescent pigment is probably bound up with the structure of pyocyanin and its formation by the bacterial cell. If the opinion of Ledderhose is accepted that this pigment is a derivative of the anthracene group, it is possible that no apparent relation exists. The fluorescent bacteria produce a green diffusible pigment along with which there is a large amount of ammonia. The pigment itself is probably basic in character.

SUMMARY

1. Of a series of 100 strains of fluorescent bacteria, isolated from water, all cultures correlate with regard to the production of fluorescent pigment, (produced very profusely when the bacteria are grown in Frankel's solution), formation of acid in glucose broth, absence of diastasic action upon potato starch, negative indol formation and non-fermentation of sucrose and lactose.

2. These cultures, when studied according to the group number as expressed on the descriptive chart of the Society of American Bacteriologists, fall into 27 groups.

3. The fluorescent bacteria are about evenly divided with regard to gelatin liquefaction. The test is not delicate when applied to this group and requires further study.

4. Four of the 100 strains are spore formers. This characteristic is recognized as one which logically separates a bacterial species.

5. With the exception of the four spore formers, the fluorescent bacteria in water, as indicated by this study, constitute a homogeneous group of bacteria, difference appearing only in regard to gelatin liquefaction, formation of hydrogen sulfide and perhaps fermentation of glycerol.

It is a pleasure to acknowledge my indebtedness to Prof. Edward Bartow and Prof. H. A. Harding for their personal interest and valuable suggestions throughout this work.

REFERENCES

BABES 1889 Note sur quelques matières colorantes et aromatiques produites par le bacille pyocyanique. Compt. Rend. Soc. Biol., **41**, 438–439.

BELLI 1902 Chemische microscopische und bakteriologische Untersuchungen ueber den Hagel. Cent. f. Bakt., II Abt., **8**, 445–446.

BEYERINCK 1891 Die Lebensgeschichte einer Pigment Bakteria. Bot. Zeit., **49**, 705.

BOLAND 1899 Ueber pyocyanin den blauen Farbstoff des *Bacillus pyocyaneus*. Centralbl. f. Bakt., **25**, 897–902.

BUCHANAN 1915 Nomenclature of the Coccaceae. Jour. Inf. Dis., **17**, 528–541.

BURGE AND NEILL 1915 The comparative rate at which fluorescent and non-fluorescent bacteria are killed on exposure to ultraviolet light. Am. Jour. Physiol., **38**, 399–403.

BURGER 1914 Formation of Hydrogen Sulfid from Cystin by Bacteria. Arch. f. Hyg., **82**, 201–211.

CHAMOT AND REDFIELD 1915 Studies on culture media employed in bacteriological examination of water. Jour. Am. Chem. Soc., **37**, 1606–1630.

COMMITTEE 1898 Procedures recommended for the study of bacteria with special reference to greater uniformity in the description and differentiation of species. Being the report of a committee of bacteriologists to the committee on purification of water supplies of the American Public Health Association. Am. Pub. Health Assoc. Proc., **23**, 60–100, 1898.

CONN, H. W. 1906 Classification of Dairy Bacteria. Ann. Rep. Conn. Agr. Exp. Station, 91–203.

CONN, H. W. 1910 Practical Dairy Bacteriology. Orange Judd & Co., 217.

CONN, H. J. 1915 A study of *B. subtilis* by the classification card. Science, N. S., **41**, 618.

CORNWALL 1915 Classification of bacteria for the purpose of water analysis. Indian. Jour. Med. Res., **2**, 352–368. Original not seen. Reviewed in Chem. Abstracts, **9**, 328.

EDSON AND CARPENTER 1912 The green fluorescent bacteria occurring in maple sap. Vermont Agr. Exp. Station. Bull. No. 167, 521–599.

EISENBERG 1914 Untersuchungen über die Variabilität der Bakterien. Centralbl. f. Bakt. I Abt., **73**, 466–470.

FORDOS 1863 Recherches sur la matière colorante des suppurations bleues-pyocyanine et pyoxanthose. Compt. Rend., **56**, 1128–1131.

FULLER AND JOHNSON 1899 On the differentiation and classification of water bacteria. Jour. Exp. Med., **4**, 609–626.

GAGE AND PHELPS 1902 On the classification and identification of bacteria with a description of the chart system in use at the Lawrence Experiment Station for records of species. Am. Pub. Health Assoc. Proc., **28**, 494–505.

GESSARD 1890 Des races du bacille pyocyanique. Ann. de l'Inst. Past., **5**, 65.

GRIFFON 1909 Sur le rôle des bacilles fluorescent de Flügge en pathologie vegetale. Compt. Rend. Acad. Sci., **149**, 51–53.

HARDING 1910 The constancy of certain physiological characters in the classification of bacteria. N. Y. (Geneva) Agr. Exp. Station, Tech. Bull. No. 13.

HARDING, H. A. AND PRUCHA, M. J. 1908 The bacterial flora of Cheddar cheese. N. Y. (Geneva) Agr. Exp. Station, Tech. Bull. No. 8.

HARDING, MORSE AND JONES 1909 The bacterial soft rot of certain vegetables. N. Y. (Geneva) Agr. Exp. Station, Tech. Bull. No. 11.

HARRISON 1898 Bacterial content of hail stones. Bot. Gaz., **25,** 211–214.

HASTINGS 1904 The action of various classes of bacteria on casein as shown by milk agar plates. Centralbl. f. Bakt., II Abt., **12,** 590–593.

JOHNSTON, W. 1894 On the grouping of water bacteria. Am. Pub. Health Assoc. Proc., **20,** 445–449.

JORDAN 1899 The production of fluorescent pigment by bacteria. Bot. Gaz., **27,** 19–36.

JORDAN, E. O. 1903 The kinds of bacteria found in river water. Jour. Hyg., **3,** 1–27.

KRAUSE 1900 Beiträge zur Kenntnis des *Bacillus pyocyaneus*. Centralbl. f. Bakt., I Abt., **27,** 769–775.

KRUSE 1896 The fluorescent group of bacilli. Die Mikroorganismen. Flugge 3rd edition, Vol. II, 289.

LAUBACH 1916 Studies on aerobic spore-bearing non-pathogenic bacteria: Spore-bearing organisms in water. Jour. Bact., **1,** 505–506.

LEDDERHOSE 1888 Ueber den blauen Eiter. Deut. Ztschr. f. Chir., **28,** 201.

MUIR AND RICHIE 1913 Manual of Bacteriology, p. 110.

NIEDERKORN 1900 Vergleichende Untersuchungen über die verschiedenen Varietäten des *Bacillus pyocyaneus* und des *Bacillus fluorescens liquefaciens*. Centralbl. f. Bakt., I Abt., **27,** 749–750.

NOGIER, DUFORT AND DUJOL 1913 Contribution a l'etude des pigments du *bacillus pyocyaneus*. Jour. Physiol. path. gen., **15,** 633–635.

RAHN AND HARDING 1914 Die Bemühungen zur einheitlichen Beschreibung der Bakterien in Amerika. Centralbl. f. Bakt., II Abt., **42,** 385–393.

REDFIELD 1912 A study of hydrogen sulfid production by bacteria and its significance in the sanitary examination of water. Thesis. Cornell University.

RETTGER AND SHERRICK 1911 Studies on bacterial variation. Jour. Med. Res., **19,** (N. S.) 265–284.

ROGERS AND DAVIS 1912 Methods of classifying the lactic acid bacteria. U. S. Dept. Agr., Bur. An. Ind. Bull. No. 154.

RUZIKA 1898 *Bacillus pyocyaneus* and Bac. *fluorescens liquefaciens*. Arch. f. Hyg., **34,** 140. Experimentelle Studien ueber die Variabilitat wichtigen Charaktere des *B. pyocyaneus* und *B. fluorescens liquefaciens*. Centralbl. f. Bakt., **24,** 11–17.

SASAKI AND OTSUKA 1912 Experimentelle Untersuchungen über die Schwefelwasserstoffentwicklung der Bakterien aus Cystin und sonstigen Schwefelbindungen. Biochem. Ztschr., **39,** 208–215.

SCHMELCK 1888 Steigerung des Bakteriengehaltes im Wasser während des Schneeschmelzens. Centralbl. f. Bakt., **4,** 195–199.

SULLIVAN 1905 Synthetic culture media and the biochemistry of bacterial pigments. Jour. Med. Res., **14,** 109–160.

TANNER 1917 Studies on bacterial metabolism of sulfur. Jour. Bact., **1,** 585–593.

THONI 1911 Biologische Studien über Limonaden. Centralbl. f. Bakt., II Abt., **29**, 616–643.

THUMM 1905 Beiträge sur Biologie der fluoreschirenden Bakterien. Arb. a. d. Bakt. Inst. der Hochschule. **1**, 291–377. 1895 Original not seen. Reviewed by Smith. Bacteria in Relation to Plant Diseases. Vol. 1, 238.

WARD, M. 1897 Fifth report to the Royal Society Water Research Committee. Proc. Roy. Soc. London, **61**, 415–423.

WASSERZUG 1887 Sur la formation de la matière colorante chez le Bacillus pyocyaneus. Ann de l'Inst. Pasteur., **1**, 581–591.

WINSLOW 1914 The characterization and classification of bacterial types. Science, N. S., **39**, 77–90.